IOWA STATE COLLEGE

JOURNAL OF SCIENCE

Published on the first day of October, January, April, and July

All manuscripts submitted should be addressed to G. F. Stewart, Agricultural Experiment Station, Iowa State College, Ames, Iowa.

All remittances should be addressed to The Iowa State College Press, Press Building, Ames, Iowa.

Single Copies: $1.25 (Except Vol. XVII, No. 4—$2.00). Annual Subscription: $4.00; in Canada $4.25; Foreign $4.50.

Entered as second-class matter January 16, 1935, at the post office at Ames, Iowa, under the act of March 3, 1879.

GROWTH OF THE WHITE BASS, *LEPIBEMA CHRYSOPS* (RAFINESQUE), IN CLEAR LAKE, IOWA [1]

WILLIAM M. LEWIS[2]

From the Entomology and Economic Zoology Section, Iowa Agricultural Experiment Station, the Industrial Science Research Institute, and the Iowa State Conservation Commission

Received September 27, 1949

Clear Lake, in Cerro Gordo County, north central Iowa, is one of the most important natural fishing lakes in the state. Bailey and Harrison (1) published a description of the lake and its fish populations. Growth studies have been made on the yellow bass, *Morone interrupta* Gill (8), and on the yellow pikeperch, *Stizostedion v. vitreum* (Mitchill) (2). The present study summarizes the data which have been collected on the white bass, *Lepibema chrysops* Rafinesque.

Bailey and Harrison (1) reported that white bass were abundant in Clear Lake in 1941, 1942, and 1943, but collections in 1947 by Robert E. Cleary and in 1948 by James G. Sieh indicated that the species was relatively scarce during the time they were collecting. The yellow bass is reported to have been abundant in all five years.

Age and growth studies were made by the scale method in the conventional manner. Due to the small number of specimens, the lack of key scales, and the limited size range of the fish, a body-scale relationship was not made. Calculations were based upon the relationship given by Van Oosten (13), which was a straight line with its origin at 24 millimeters and having a slope of 1.08. Sigler (11) determined the relationship of white bass from Spirit Lake to be similar.

Since upon separate analysis the growth rates of the males and females were approximately the same and showed no consistent differences, the data for both sexes were combined. The average calculated total lengths in inches at the end of each year of life were 6.0, 10.5, 12.9, 14.0, 14.8, and 15.5 for the first through the sixth year, respectively (Table 1). The growth rate in Clear Lake was apparently fairly rapid when compared with the growth of white bass in other waters.

Considering the small sample sizes, the various year classes were quite well represented (Table 3). Among the 103 fish taken in 1941, 1942, and 1943, the 1939 year class was best represented, making up 43 per cent of the total number. The 1937, 1938, and 1940 classes were

[1] Journal Paper No. J-1666 of the Iowa Agricultural Experiment Station, Ames, Iowa, Project No. 765, and the Industrial Science Research Institute (Project No. 46) of Iowa State College; in cooperation with the Iowa State Conservation Commission.

[2] The writer wishes to express his appreciation to Dr. Reeve M. Bailey, Mr. Harry M. Harrison, Mr. Robert E. Cleary, and Mr. James Sieh for permission to use the scales and data upon which this paper is based; and to Dr. Kenneth D. Carlander, who supervised the analysis of the data.

TABLE 1

AVERAGE CALCULATED LENGTHS AT EACH YEAR OF LIFE OF 118 WHITE BASS COLLECTED FROM CLEAR LAKE, 1941 TO 1948

Age Group	Number Examined	Standard Length in Mm. at Capture	Average Calculated Length at Each Annulus					
			1	2	3	4	5	6
0	1	135						
I	2	131	81					
II	13	242	123	198				
III	47	275	119	206	255			
IV	45	281	115	204	248	273		
V	8	289	112	202	245	269	286	
VI	2	305	124	219	255	280	294	302
Mean standard lengths, millimeters			117	204	251	273	288	302
Equivalent total length inches*			6.0	10.5	12.9	14.0	14.8	15.5
Annual increment, standard length, millimeters			117	87	46	25	17	8
Standard deviation of standard lengths			12.9	15.2	11.4	16.4	29.2	8.5

*Total length equals 1.306 standard lengths according to measurements on 105 specimens in the standard length range, 112 to 313 millimeters.

TABLE 2

COMPARISON OF REPORTED GROWTH RATES OF WHITE BASS FROM VARIOUS LOCALITIES*

Locality	Number of Specimens	Calculated Total Length in Inches at End of Each Year of Life								
		1	2	3	4	5	6	7	8	9
Minnesota (3)†, ‡	69	4.1	6.8	9.5	11.1	12.6				
Lake Erie (13)	1,835	4.7	8.2	10.9	12.4	13.2	13.6	14.0		
Spirit Lake, Iowa (11)	569	5.0	9.7	12.6	14.0	14.5	14.9	15.3	15.6	16.2
Clear Lake, Iowa	118	6.0	10.5	12.9	14.0	14.8	15.5			
Okoboji Lake, Iowa§	6	5.1	10.5	13.2	14.0					
Storm Lake, Iowa (12)	100	5.6	11.4	14.1	15.5					
Wheeler Reservoir, Ala. (7)†,‡	346	7.1	11.6							
Clinch River, Tenn. (5)†	133	9.0	11.8							
Clinch River, Tenn. (4)‡	37	9.4								

*Additional growth data on white bass have been published by Roach (10), The Ohio Division of Conservation (9), and Frey and Vike (6).

†Originally calculated from zero origin but here adjusted to 24 millimeter origin for purposes of comparison.

‡Originally stated in terms of fork length but here converted to total length, by formula given by Van Oosten (13).

§Six fish collected in 1941 by Max Davis.

TABLE 3

NUMBERS AND LENGTHS OF WHITE BASS IN EACH SAMPLE BY YEAR AND AGE CLASS, CLEAR LAKE, 1941–48

Date Collected	Age Class	Year Class	Number Specimens	Standard Length in Mm.	
				Mean	Range
Oct. 1941	0	1941	1	135	.....
	II	1939	12	241	229–251
	III	1938	4	251	242–269
	IV	1937	13	286	270–302
	V	1936	1	292	.....
	VI	1935	1	290	.....
April 1942	III	1939	9	253	236–269
	IV	1938	10	275	245–289
	V	1937	4	289	268–312
Oct. 1942	III	1939	8	276	257–291
	IV	1938	5	301	285–312
July 1943	III	1940	21	278	244–313
	IV	1939	14	276	253–299
June, July and Aug. 1947	I	1946	1	97	.....
	II	1945	1	249	.....
	III	1944	5	274	249–325
	IV	1943	1	284	.....
	V	1942	1	284	.....
	VI	1941	1	320	.....
June, July and Aug. 1948	I	1947	1	165	.....
	IV	1944	2	267	267–267
	V	1943	2	290	229–351

about equal in abundance. Only one fish each from the 1935, 1936, and 1941 classes occurred in the samples. The first two of these classes had probably largely disappeared by the time the study was undertaken. Sigler (11) found the 1941 year class abundant in Spirit Lake but not in Storm Lake (12). This year class apparently was poorly represented in Clear Lake.

TABLE 4

WEIGHTS AND COEFFICIENTS OF CONDITION (K) OF 71 WHITE BASS FROM CLEAR LAKE

Age Group	Date Collected	No. of Fish	T.L. Inches	S.L. Mm.	Av. Wt. Ounces	Av. Wt. Grams	K
III	Apr. 15-29'42	9	12.3	250	14.9	422	2.8
III	July 22-24'43	21	13.5	274	22.2	630	2.9
III	Oct. 1-3'42	8	13.6	276	23.8	675	3.2
IV	Apr. 15-29'42	10	13.6	275	20.7	587	2.8
IV	July 22-24'43	14	13.6	276	21.2	603	2.8
IV	Oct. 1-3'42	5	14.8	301	28.5	808	3.0
V	Apr. 15-29'42	4	14.2	289	24.4	702	2.9

In their third year of life the white bass ranged from a little less than a pound to about 1½ pounds. In their fourth year they ranged from about 1⅓ pounds to 1¾ pounds (Table 4).

The coefficient of condition, K, was median in position when compared to values reported from other areas (Table 5), but caution should be used in making such a comparison since Sigler (11) clearly demonstrated a systematic increase in K with an increase in the length of the specimen. In the length range 39 to 346 millimeters, he reported an upward trend of K from 2.121 to 3.113. There did not appear to be any consistent change in K values with increase in length of the white bass from Clear Lake.

TABLE 5

COMPARISON OF COEFFICIENT OF CONDITION OF WHITE BASS COLLECTED FROM VARIOUS LOCALITIES

Locality	No. of Fish	Average K Value
Lake Erie (13)	3,510	2.4
Wheeler Reservoir (7)	522	2.5
Clinch River, Tenn. (5)	133	2.7
Clear Lake, Iowa	71	2.7
Clinch River, Tenn. (4)	589	2.8
Storm Lake, Iowa (12)	98	2.8
Spirit Lake, Iowa (11)	1,459	2.9

LITERATURE CITED

1. BAILEY, REEVE M. AND HARRY M. HARRISON, JR.
1945. The fishes of Clear Lake, Iowa. Iowa State Coll. Jour. Sci., 20:57–77.

2. CLEARY, ROBERT E.
1949. Life history and management of the yellow pikeperch, *Stizostedion v. vitreum* (Mitchill), of Clear Lake, Iowa. Iowa State Coll. Jour. Sci., 2:195–208.

3. EDDY, SAMUEL AND KENNETH CARLANDER
1939. Growth of Minnesota fishes. Minnesota Conservationist, June, pp. 8-10.

4. ESCHMEYER, R. WILLIAM
1944. Fish migration in Clinch River below Norris Dam, Tennessee. Jour. Tenn. Acad. Sci., 19:31–41.

5. ——— AND D. E. MANGES
1945. Fish migrations into the Norris Dam tailwater. Jour. Tenn. Acad. Sci., 20:92–97.

6. FREY, DAVID G. AND LAWRENCE VIKE
1941. A creel census on Lakes Waukesa and Kegonsa, Wisconsin, 1939. Trans. Wis. Acad. Sci., Arts, and Letters, 33:339–62.

7. HOWELL, HENRY H.
1945. The white bass in TVA waters. Jour. Tenn. Acad. Sci., 20:41–48.

8. LEWIS, W. M. AND K. D. CARLANDER
1948. Growth of the yellow bass, *Morone interrupta* Gill, in Clear Lake, Iowa. Iowa State Coll. Jour. Sci., 22:185–95.

9. OHIO DIVISION OF CONSERVATION
1939. Sandusky Bay fishing survey—1938. Ohio Conservation Bull. 3:4–5.

10. ROACH, LEE S.
1943. Buckeye Lake white bass. Ohio Jour. Sci., 43:263–66.

11. SIGLER, WILLIAM F.
1949. Life history of the white bass *Lepibema chrysops* (Rafinesque) of Spirit Lake, Iowa. Iowa Agr. Exp. Sta. Res. Bull. 366:203–244.

12. ———
1949. Life history of the white bass in Storm Lake, Iowa. Iowa State Coll. Jour. Sci. 23(4):311–316.

13. VAN OOSTEN, JOHN
1942. The age and growth of Lake Erie white bass, *Lepibema chrysops* (Rafinesque). Papers Mich. Acad. Sci., Arts, and Letters, 27(1941): 307–34.

ON THE OXIDATIVE DECARBOXYLATION OF α-KETOGLUTARIC ACID[1]

SAMUEL J. AJL AND C. H. WERKMAN

Bacteriology Section, Iowa Agricultural Experiment Station, Ames, Iowa

Received October 3, 1949

This communication deals with the bacterial enzyme involved in the oxidative decarboxylation of α-ketoglutaric acid to succinate and CO_2. This reaction is of particular significance because of its role in heterotrophic assimilation of carbon dioxide by bacteria. It is likely that the carboxylation of succinate by Ochoa's (1) cat heart preparation involves the same or similar enzyme. Inorganic phosphate, adenosine triphosphate (ATP), and magnesium are involved in the action of the bacterial enzyme.

METHODS

Preparation of the enzyme system has been described (2). Iowa State stock cultures of *Escherichia coli* were grown for 16–18 hrs. at 30°C. in a medium containing 0.8 per cent glucose, 0.4 per cent $(NH_4)_2SO_4$, 0.8 per cent KH_2PO_4, 0.2 per cent yeast extract and 10 per cent tap water at an initial *p*H of 7.0. Somewhat better preparations have been obtained when the organism was grown on a medium in which 1 per cent tryptone replaced the yeast extract. The cells were then harvested and ground with powdered glass and subsequently extracted with distilled water or M/15 phosphate buffer solution (3). One and a half ml. of eluting liquid was used per gram of cells.

Dialysis of the enzyme preparations were carried out in a collodion bag against distilled water at approximately 1°C. for 90 to 120 minutes. The dialyzing apparatus consisted of two parts: (a) a motor rotating a glass rod to which the collodion bag was attached with heavy thread; the glass rod extended into (b) a dialyzing chamber at an angle of 45°C.; the capacity of the dialyzing chamber was approximately 10 liters, containing distilled water and ice cubes.

Lyophilized preparations—Cell-free preparations were transferred into a 500 ml. round-bottom flask and frozen while rotating the flask in an acetone-dry ice bath. The flask containing the frozen culture was evacuated by use of a vacuum pump. The evacuation of air resulted in sublimation of the ice in the frozen suspension. The water vapors were extracted by continuous suction and condensed and frozen in another flask which was held in an acetone-dry ice bath.

The Barcroft-Warburg technique was used in all experiments. The

[1] Journal Paper No. J-1632 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 746.

total volume of reactant varied from 2.3 to 4 ml. Endogenous activity is negligible for dialyzed cell-free preparations. Somewhat higher endogenous values are obtained on addition of such substances as ATP or Mg. Extreme caution was therefore exercised in setting up proper endogenous controls and deducting endogenous values. It should be noted, however, that in no case was the endogenous value above 5 per cent of the experimental.

When whole cells were employed they were either centrifuged, washed several times with distilled water and used immediately, or lyophilized immediately after centrifuging and stored at approximately 5°C.

EXPERIMENTAL

In the presence of dialyzed *E. coli* suspensions and malonate the following equation obtains:

(1) $COOHCH_2CH_2COCOOH + 1/2\ O_2 \rightleftharpoons COOHCH_2CH_2COOH + CO_2$

Since Stumpf, *et al.* (4) obtained from animal tissues enzyme preparations which in the presence of cocarboxylase and magnesium catalyzed the anaerobic decarboxylation of α-ketoglutarate to succinic semialdehyde, its oxidative decarboxylation (Equation 1) may be a result of the following two reactions:

(2) $COOHCH_2CH_2COCOOH \longrightarrow COOHCH_2CH_2CHO + CO_2$

(3) $COOHCH_2CH_2CHO + 1/2\ O_2 \longrightarrow COOHCH_2CH_2COOH$

This possibility was investigated with the *E. coli* preparation. If the aldehyde of succinic acid is an intermediate, one mole of CO_2 should be liberated for each mole of α-ketoglutarate under anaerobic conditions. In the presence of oxygen-free nitrogen the cell-free preparation of *E. coli* or the intact cells, whether freshly harvested or lyophilized and stored, fail to catalyze the anaerobic decarboxylation of α-ketoglutarate with or without malonate (Table 1). This fact also excludes the possibility of α-ketoglutaric acid dismutating as reported by Weil-Malherbe (5) for animal tissue.

Components of the enzyme system—It is at present difficult to ascertain whether one or more enzymes are involved in the oxidative

TABLE 1

ANAEROBIC DECARBOXYLATION OF α-KETOGLUTARIC ACID

(All of the preparations oxidized α-ketoglutaric acid in the presence of oxygen.)

Enzyme Preparation	Cell-free Enzyme Preparation*	Washed Suspension of Cells†	Lyophilized Cells‡
CO_2 evolved μl.....	2	17	9

*Total volume of reactants 2.3 ml. anaerobic; 0.0043 M α-ketoglutaric acid, 0.043 M PO_4 buffer, *p*H 7.0, temp. 30.4°C., 1.0 cc. of cell-free juice.

†Total volume 2.0 ml. anaerobic; 0.0125 M α-ketoglutaric acid, 0.043 M PO_4 buffer, *p*H 7.0, temp. 30.4°C., 10 per cent suspension of cells (0.5 cc.).

‡Same as 2. Lyophilized cells were used at the rate of 30 mg./cup.

decarboxylation of the keto acid. Some of the components involved have been identified by activating the apoenzyme after dialysis had led to inactivity. Inorganic phosphate, adenosine triphosphate and magnesium ions replace all that has been removed by dialysis for three hours against ice-cold distilled water (Table 2). The addition of biotin to the enzyme system containing phosphate, magnesium and ATP increases the oxygen uptake from 204 μl. as given in Table 2 to 252 μl., a value almost equal to the oxygen uptake in the presence of boiled juice. Since malonate is not a perfect block for the oxidation of succinate arising from α-ketoglutarate, it is difficult to determine accurately whether this vitamin is actually involved in the oxidative decarboxylation of the keto acid or in some subsequent step. Since experimental data have been obtained indicating a role of biotin in succinic acid oxidation, little attention may be given to the increased oxygen uptake in the presence of biotin or boiled juice as far as α-ketoglutaric acid dehydrogenase is concerned. The enzyme components are therefore phosphate, magnesium, and ATP.

Attempts to replace some of the above-mentioned components of α-ketoglutaric dehydrogenase indicate that manganese and to a less extent nickel can be used to replace magnesium, whereas other divalent ions such as zinc and cobalt are inhibitory even in minute concentrations (Table 3). It is doubtful whether nickel has any physiological function, but its partial replacement of magnesium is significant from a theoretical standpoint in so far as it may indicate a rather general function of the divalent ions in metabolism.

The question of zinc was further investigated. Barron and Kalnitsky (6) were able to inhibit succinoxidase by heavy metals such as zinc and reactivate the enzyme by the addition of dithiols. A similar experiment was set up using glutathione and cysteine after poisoning the α-ketoglutaric dehydrogenase with $ZnCl_2$. The results are given in Table 4. Since glutathione nearly completely reverses the inhibition due to zinc, the inhibition produced by this heavy metal may be the result of tieing up —SH groups of the protein moiety of the enzyme system involved.

Stumpf, *et al.* (4) reported the involvement of diphosphothiamine in the oxidation of both α-ketoglutarate and pyruvate. Under our conditions thiamine pyrophosphate has no function (Table 5). Adenylic acid will not replace ATP in bacterial α-ketoglutaric acid dehydrogenase (Table 5).

The possible route of hydrogen transfer was also investigated. The function of the C_4 dicarboxylic acids as hydrogen carriers in metabolic systems as postulated by Szent-Györgyi is questioned (Potter, 7) particularly in bacterial respiration. However, in our experiments a notable increase in oxygen uptake was observed when fumarate and malate were added to α-ketoglutaric acid (Table 6). Ochoa (1) reports no such increase. Our results are of particular value since malate and fumarate were not appreciably attacked by the enzyme preparation and yet when added to the keto acid increased the amount of oxygen utilization.

TABLE 2

SOME COMPONENTS OF α-KETOGLUTARIC ACID DEHYDROGENASE

(Total volume of reactants varied from 2.3 to 3.1 ml. Expt. 1—0.0043 M α-ketoglutaric acid, 0.043 M PO_4 buffer *p*H 7.0, 0.0043 M $MgCl_2$, 0.001 M ATP, 1 cc. of dialyzed juice Expt. 2—0.0033 M α-ketoglutaric acid, 0.033 M PO_4 buffer, *p*H 7.0, 0.0033 M $MgCl_2$, 0.001 M ATP 1 cc. of dialyzed juice and 0.5 cc. of boiled [10 min.] juice. Temp. 30°C.] Time, 3 hrs. In both experiments 0.003 M malonate was used. Appropriate enzyme blanks were deducted from substrate values.)

	Exp. No.	Additions to Dialyzed Juice						
		None	$PO_4^{\equiv}$	Mg^{++}	ATP	$PO_4^{\equiv} + Mg^{++}$	$PO_4^{\equiv} + Mg^{++}$ + ATP	$PO_4^{\equiv} + Mg^{++}$ + ATP + boiled juice
O_2 uptake μl.	1	−3	57	73	143			
						74	204	260

TABLE 3

REPLACEMENT OF MAGNESIUM BY DIVALENT IONS

(Total volume of reactants 2.3 ml. The various ions were added to 0.0043 M α-ketoglutarate, 0.0033 M malonate, and 1 cc. of dialyzed juice.)

Ions	Mg^{++}	Ni^{++}		Co^{++}		Zn^{++}		Mn^{++}
	0.0043 M	0.0004 M	0.0043 M	0.0004 M	0.0043 M	0.0004 M	0.0043 M	0.0043 M
O_2 uptake μl.	120	41	−3	−28	−49	−24	−74	102

TABLE 4

INHIBITION (BY $ZnCl_2$) AND REACTIVATION (BY GLUTATHIONE) OF α-KETOGLUTARIC ACID DEHYDROGENASE

(Total volume 2.8 ml. 0.0036 M α-ketoglutarate, 0.0021 M malonate, 0.0036 M PO_4 buffer, *p*H 7.0, 0.0003 M $ZnCl_2$, 0.001 M glutathione, 0.001 M cysteine. NaOH in center well. 1 cc. of *E. coli* juice. Temp. 30.4°C.)

Inhibitor	Activator	Oxygen Uptake μl.
.....	.....	100
Zn.....	.....	39
Zn.....	glutathione	85
Zn.....	cysteine	−32

Other hydrogen carriers have also been determined. Poisoning of the entire system by cyanide indicates that heavy metal catalysis is involved (Table 7). Monoiodoacetate also completely inhibits the enzyme system, presumably by blocking some dehydrogenase, and the inhibition due to fluoride suggests that a phosphorylated compound (or compounds) plays a part. The latter result is expected since the activity of dialyzed juice is increased by the addition of phosphate as previously noted.

Properties of enzyme—The activity between α-ketoglutaric acid dehydrogenase obtained from *E. coli* and substrate is moderate. The amount of oxygen uptake may vary from 7 to 150 μl. in the first hour.

The enzyme is relatively stable over a period of weeks when kept frozen in the icebox. Lyophilizing the juice results in loss of activity. When the oxidation is carried out in an atmosphere of oxygen, the rate of the reaction is increased. Optimum activity is at *p*H of approximately 7.0.

DISCUSSION

Increase of the activity of the dialyzed juice by phosphate strongly supports the validity of the suggested mechanism for the reaction studied (2). The first step in this reaction is apparently a phosphorylation of α-ketoglutaric acid. This phosphate is then utilized for the formation of succinyl phosphate, an energy-rich compound and a potential source of energy for the formation of adenosine triphosphate. Such oxidative decarboxylations are of extreme importance to the total economy of cell since they offer means by which the organisms can utilize the energy produced by the oxidation of the substrate.

The bacterial enzyme does not decarboxylate α-ketoglutaric acid under anaerobic conditions. This makes our enzyme distinct from the one reported by Stumpf, *et al.* (4) and Weil-Malherbe (5). However, this keto acid replaces CO_2 under anaerobic conditions, which apparently indicates that its function under such conditions concerns transamination or amination (8).

The fact that nickel can partially replace magnesium or manganese

TABLE 5

FUNCTION OF COCARBOXYLASE, ADENOSINE TRIPHOSPHATE AND ADENYLIC ACID IN α-KETOGLUTARIC ACID DEHYDROGENASE

(Total volume of reactants 3.0 ml. 0.0033 M α-ketoglutaric acid, 0.033 M PO_4 buffer, *p*H 7.0; 0.0033 M $MgCl_2$, 0.001 M ATP, 25γ per cup cocarboxylase, 0.013 M adenylic acid and 0.033 M malonate. 1 cc. dialyzed juice. Temp. 30.4°C.)

		Additions to Dialyzed Juice						
	Exp. No.	None	Mg^{++}	Cocarboxylase	Cocarboxylase + Mg^{++}	$PO_4^{\equiv}$	ATP + Mg^{++} + $PO_4^{\equiv}$	AA + Mg^{++} + $PO_4^{\equiv}$
O_2 uptake μl.	1	−3	73	−10	75	57		
							204	64

TABLE 6

EFFECT OF C_4 DICARBOXYLIC ACIDS ON OXIDATION OF α-KETOGLUTARATE

(Total volume 2.3 ml. 0.054 M PO_4 buffer, *p*H 7.0. 0.0017 M malonate. 1 cc. *E. coli* juice. Temp. 30.4°C.)

Substrate	Succinate	Fumarate	Malate	α-Ketoglutarate	α-Ketoglutarate + Fumarate	α-Ketoglutarate + Malate
Concentration, M	0.010	0.010	0.010	0.010	0.010 + 0.0017	0.010 + 0.0017
Oxygen uptake μl	80	29	−5	7	52	39

TABLE 7

EFFECT OF INHIBITORS ON THE OXIDATION OF α-KETOGLUTARIC ACID BY *E. coli* JUICE

(Total volume 2.8 ml. 0.0088 M a-ketoglutarate, 0.0036 M malonate, 0.017 M PO_4 buffer, *p*H 7.0. 1 cc. *E. coli* juice. Temp. 30.4°C.)

Inhibitor	Concentration	Point of Inhibition	Effect
Cyanide	0.0008 M	Cytochrome	Completely inhibits
Iodoacetate	0.008 M	Dehydrogenases	Completely inhibits
Fluoride	0.008 M	Phosphate esters	Partial inhibition

in this reaction is of theoretical importance only. It may be used to support the hypothesis that when divalent ions are involved in certain enzyme systems, their function is merely to bind the protein and prosthetic group together due to their divalency. Many exceptions, however, are noted. Zn, for instance, although a divalent metal, does not function in a similar manner, since it apparently ties up —SH groups of the protein moiety of the enzyme. It is very difficult to decide the kind of linkage formed between the heavy metal and the —SH groups of the protein molecule. However, the mechanism suggested by Barron and Kalnitsky (6) in which two —SH groups combine with the heavy metal (e.g., Protein $\begin{matrix}/S\backslash \\ \backslash S/\end{matrix}$ metal to form cyclic compounds is possible.

The dehydrogenation of α-ketoglutaric acid probably proceeds through a series of hydrogen carriers. The evidence that we have obtained that fumarate and malate are involved in the dehydrogenation of the keto acid should be stressed, for it places emphasis on the C_4 dicarboxylic acids in bacterial metabolism.

SUMMARY

An enzyme involved in the oxidation of α-ketoglutaric acid by a cell-free extract of *Escherichia coli* has been described and the components identified.

LITERATURE CITED

1. OCHOA, S. J.
1944. Jour. Biol. Chem. 155:87.

2. AJL, S. J. AND C. H. WERKMAN
1948. Proc. Natl. Acad. Sci. 34:491.

3. KALNITSKY, G., M. F. UTTER AND C. H. WERKMAN
1945. Jour. Bact. 49:595.

4. STUMPF, P. H., K. ZARUDNAYA AND D. E. GREEN
1947. Jour. Biol. Chem. 167:817.

5. WEIL-MALHERBE, H.
1937. Biochem. Jour. 31:2202.

6. BARRON, G. E. S. AND G. KALNITSKY
1947. Biochem. Jour. 41:346.

7. POTTER, V. R.
1940. Jour. Biol. Chem. 134:417.

8. AJL, S. J. AND C. H. WERKMAN
1949. Jour. Bact. 57:579.

FISHERIES INVESTIGATIONS ON TWO ARTIFICIAL LAKES IN SOUTHERN IOWA II. FISH POPULATIONS[1]

WILLIAM M. LEWIS[2]

Department of Zoology and Entomology, Iowa State College

Received October 13, 1949

Red Haw and East Lakes near Chariton, Iowa, provide unusual opportunities for fisheries investigations. Each lake is about eighty acres in area and has a maximum depth of twenty-five to thirty feet. The two reservoirs are in the same valley, and their waters have similar chemical and biological characteristics except for the periodic copper sulphate treatment of East Lake. Red Haw is a state-owned lake used primarily for recreational purposes, whereas East Lake is primarily a city water supply reservoir with recreation and fishing purposes in a secondary position. A more complete description of the two lakes and their vegetation has already been published (29).

During the field investigations, March 26 to September 22, 1948, the following species of fish were taken from both lakes:

Western golden shiner, *Notemigonus crysoleucas auratus* (Rafinesque)
Northern black bullhead, *Ameiurus melas melas* (Rafinesque)
Northern brown bullhead, *Ameiurus nebulosus nebulosus* (Le Sueur)
Northern yellow bullhead, *Ameiurus natalis natalis* (Le Sueur)
Yellow perch, *Perca flavescens* (Mitchill)
Largemouth black bass, *Micropterus salmoides* (Lacepede)
Warmouth, *Chaenobryttus coronarius* (Bartram)
Green sunfish, *Lepomis cynellus* (Rafinesque)
Bluegill, *Lepomis macrochirus macrochirus* (Rafinesque)
White crappie, *Pomoxis annularis* (Rafinesque)
Black crappie, *Pomoxis nigro-maculatus* (Le Sueur)

The fish populations in the two lakes appeared to be quite similar

[1] Iowa Cooperative Fisheries Research Unit, Project No. 42, sponsored by the Industrial Science Research Institute of Iowa State College and the Iowa State Conservation Commission, with the cooperation of the U. S. Fish and Wildlife Service.

This paper is part of a thesis submitted to the Graduate Faculty of Iowa State College in partial fulfillment of the degree of Doctor of Philosophy, under the supervision of Dr. Kenneth D. Carlander. The writer wishes to express his gratitude to Drs. H. M. Harris, G. O. Hendrickson, H. H. Knight, and E. R. Becker for encouragement and advice. The help in various capacities of R. B. Moorman, Lester Lempke, John Frazer, Joe Story, F. M. Stevenson, and J. W. Parsons was of great value. The outstanding work of T. English as a field assistant is greatfully acknowledged. Ray Smith did much to further the work. The writer is particularly indebted to his wife, Sue D. Lewis, for her able assistance in the field work and in the tremendous amount of calculating connected with the growth studies. In addition to Dr. Carlander's contributions as supervisor of the work, the writer would also like to thank him for permission to use his unpublished manuscript containing a compilation of growth data.

[2] Now Assistant Professor of Zoology, Southern Illinois University, Carbondale, Illinois.

except that black crappie was the more important crappie in Red Haw Lake and the white crappie in East Lake. The channel catfish and western golden shiner were rare in Red Haw Lake but were fairly important in East Lake. Three pumpkinseeds, *Lepomis gibbosus* (Linnaeus), were collected from East Lake. A more detailed study of the abundance of the various species and the fishing which they supported will be published later. The present paper deals with the food habits, parasites, and age and growth studies on the various species of fishes in the two lakes, except for the warmouth for which a report has already been published (30) and for the green sunfish and pumpkinseed which were taken in too small numbers for detailed study.

METHOD OF PROCEDURE

To obtain as representative a sample as possible of each species of fish, six different methods of collecting were used in both lakes

TABLE 1

FOODS FOUND IN THE STOMACHS OF THE MORE IMPORTANT

	Largemouth Bass		Bluegill		
	Red Haw	East	Red Haw		East
Standard lengths	150–192	190–387	35–125	125–182	140–167
Months of collection	5–9	6–9	5,6,9	5–6	6,8,9
Number examined	25	36	23	24	56
Stomachs empty	6	12	5	4	5
Number of stomachs with following food items:					
Filamentous algae				8	
Pondweeds			3	13	29
Wheat grains					
Snails and small clams	1		2	4	6
Angleworms				1	
Unidentified insects		5			4
Grasshoppers					7
Caddis fly larvae					
Sialis furmata larvae					
Diptera larvae			10	5	5
Diptera pupae	1				
Damsel fly naiads					
Dragonfly naiads	2				1
Mayfly naiads			2	4	
Entomostraca			7		5
Crayfish	6	3			
Fish unidentified	10	6			
Bluegill fingerlings		6			
Bluegill yearlings		2			
Bluegill adults					
Golden shiner		2			
Yellow perch 2–4″		4			
Fish eggs					8
Fish scales					
Squirrel					
Unidentified material			2		

throughout the summer. Minnow seines were effective for obtaining fingerlings of most species. A thirty-foot bag net produced good catches of all sizes of all species, but due to underwater obstructions the use of this method was limited. Hoop nets appeared to be the most effective means of taking bullheads. Basket traps constructed of one-half-inch hardware cloth consistently made good catches of crappies, warmouth, yellow perch, and small bluegill. Experimental gill nets took primarily yellow perch, golden shiner, warmouth, and crappies. Fly fishing was by far the most effective method of obtaining largemouth black bass and the larger bluegills.

All weights, measurements, and examinations of specimens were made while the fish were fresh. Scales for each fish were collected in the third row below the lateral line and even with the end of the pectoral fin. Age determinations and growth calculations were made in the usual manner using the corrected body-scale relationship where such could be determined. The methods and definitions suggested by

SPECIES OF FISH IN RED HAW AND EAST LAKES, 1948

Black Crappie		White Crappie		Yellow Perch		Channel Catfish
Red Haw	East	Red Haw	East	Red Haw	East	East
68–237	114–180	90–188	165–212	128–180	107–205	348–156
5–9	6–9	4–9	4,6,7	4–8	4–8	4,6,7,8
35	10	5	32	19	75	13
9	3	0	7	7	23	2
					1	1
						5
						1
					4	
1				1		
1			4			
9	2	2	20	8	18	
					1	
1		1		2		
			2			
2			1	1		
17	3	3	8			
			1		1	3
						1
						3
1		1				
					3	
			1		1	
4	2				8	

Hile (20) were used in the calculations of coefficients of condition and length-weight relationships.

FOOD HABITS OF THE VARIOUS SPECIES

Detailed studies of the food habits were not made, but general observations of the items of food in the stomachs were made as the specimens were examined (Table 1).

The largemouth black bass (over 150 millimeters long) apparently fed primarily upon fish and crayfish in both Red Haw and East Lakes. The warmouth (30) and the channel catfish were the only other species which fed to any extent upon fish.

Insects, particularly the dipterous larvae (*Tendipedidae, Ceratopogonidae,* and *Corethra* spp.), were stable foods for the bluegills, crappies, and yellow perch. Bluegills also fed to a considerable extent upon aquatic plants. Entomostraca were important in the diet of the crappies and to a lesser extent of the bluegills. Young fish of all species, of course, depend largely upon entomostraca.

PARASITES

Observations were made on the incidence of some of the macroscopic parasites of the fish as they were examined. Since the observations were made only in the field, the identification of certain parasites was questionable, but the data are summarized for comparative purposes (Table 2).

Particular attention is called to the *Proteocephalus ambloplitis* infestation of the largemouth black bass and the *Posthodiplostomum minimum, Saprolegnia* sp. and black grub infestations of the bluegills.

Pleroceroids of *Proteocephalus ambloplitis* occurred in the ovaries of most of the female bass examined (Table 2). The number of these worms present was sufficient to nearly fill the ovaries. Ovaries which harbored the parasite were dark and blood-stained. It is doubtful if any of the female bass collected had or would have spawned. It is known that the bass tapeworm is capable of causing sterility (24). With the high degree of occurrence and infestation existing in both Red Haw and East Lake, the production of fry might be reduced to levels below that required for the proper maintenance of the bass fisheries. Seining and field observations showed that at least some fry were produced during the summer of 1948, but unfortunately there is no reliable basis for saying whether the production was high or low.

The metacercariae of *Posthodiplostomum minimum* heavily infested the livers and to a somewhat lesser degree the hearts and kidneys of the majority of the bluegills collected from both lakes (Table 2). The organisms occurred on the surface of the heart but were scattered throughout the tissues of the liver and kidney. The degree of infestation particularly among the East Lake specimens was such that the heart was completely encased and the tissues of the liver and kidney barely retained their identity. The kidneys in some cases were swollen to three or four times their normal size.

The degree of *Posthodiplostomum minimum* infestation of the East Lake bluegills was noticeably higher than that of the Red Haw specimens. This was thought to be due to the fact that the definitive host, the great blue heron, frequented East Lake constantly throughout the summer, whereas these birds were not observed on Red Haw during the field investigations.

P. minimum is reported (24) to be capable of causing the death of its fish host, yet even under the severe infections of East Lake no

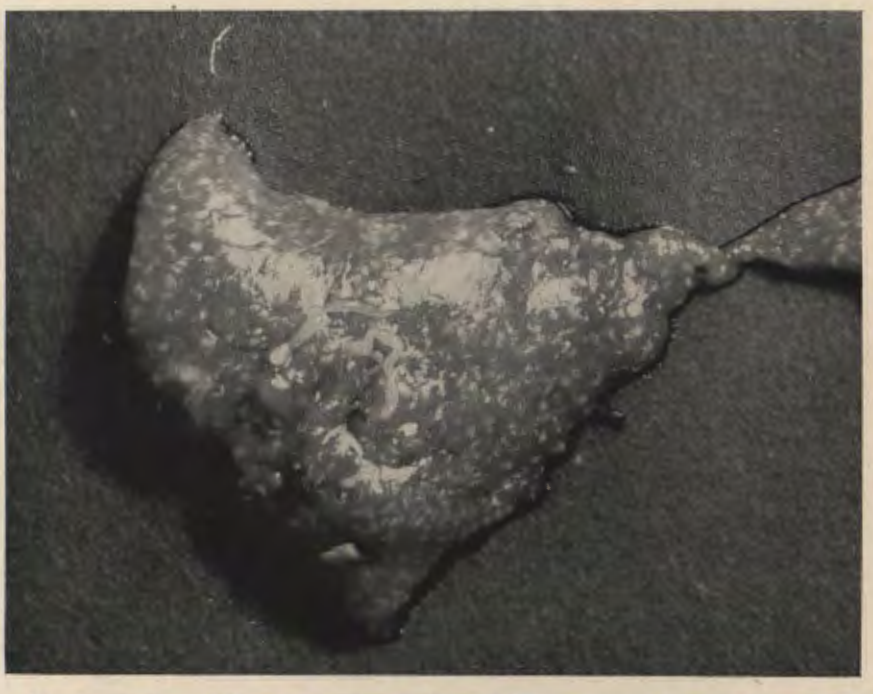

FIG. 1—Portion of liver of a bluegill from Red Haw Lake with larvae of *Posthodiplostomum minimum* and *Proteocephalus ambloplites.*

mortality was observed. Unobserved mortality could have occurred, but a careful check was made to detect it. Furthermore, mortality that occurred as a result of *Saprolegnia* sp. in Red Haw was easily detected.

During the first part of May, bait minnows held in wire cages in the lake suffered an outbreak of *Saprolegnia* sp. It appeared that the parasite was quite lethal, for the minnows died in large numbers within a day or two after the fungus mycelia became apparent. At first a few minnows were saved by separating out those not infected. Later, the water in the cages was treated with copper sulphate. Immediately after treatment the mortality of the minnows dropped to practically zero, and the disease was apparently thereafter controlled by the periodic treatments of copper sulphate. On May 16 three large bluegills on which there were areas of fungus mycelia covering a fourth or more of one side of the fish were found at Red Haw. Following this date the occurrence of

TABLE 2

PARASITES OF FISH IN RED HAW AND EAST LAKES, 1948

	Largemouth Bass		Bluegill		Black Crappie		
	Red Haw	East	Red Haw	East	Red Haw		East
Size range	93–292	196–508	36–212	74–167	under 100	100–237	80–188
Number of fish	43	42	129	127	38	102	30
Ichthyophthirius sp.	.....	.....	.....	.....	.....	.....	.....
Posthodiplostomum minimum (MacCallum)	Light infections in 37 of 39 livers and in 12 of 39 kidneys	Light infections in most livers and about 50% of kidneys	Heavy infections in128 of 129 livers, in 107 hearts and 111 kidneys	100% of livers, kidneys and hearts infected heavily	4 livers	25 livers 2 kidneys	5 livers
Clinostomum sp.	.....	.....	.....	.....	.....	.....	.....
Black grub (Strigeids)	.....	.....	17 of 129	14 of 127	.....	.....	.....
Proteocephalus ambloplites (Leidy)	In ovaries of 18 of 20 females. In mesenteries or livers of most	In ovaries of 20 of 20 females. In mesenteries or livers of most	In ovary of 1 female	In ovaries of 2, livers of 6 and mesenteries of 5	.....	In ovaries of 2	In 1 ovary
Nematodes	In 6 of 43 mesenteries	In 10 of 42 mesenteries	.....	.....	.....	2 mesenteries	.....
Leeches	Occasional on fins and upper palate	Frequent on fins and palate	On 8	.....	.....	On fins and mouth of 2	1 on palate
Gill copepods	Occasional	On 1	On 17	On 4	On 8	On 15	.....
Argulus sp.	One	.....	One	.....	.....	.....	.....

TABLE 2 (*continued*)

PARASITES OF FISH IN RED HAW AND EAST LAKES, 1948

	White Crappie		Yellow Perch		Golden Shiner	Channel Catfish	Yellow Bullhead	Brown Bullhead	Black Bullhead
	Red Haw	East	Red Haw	East	East	East	Red Haw	Red Haw	Red Haw
Size range	88–254	71–212	92–180	90–205	.....	348–756	155–289	186–212	134–321
Number of fish	23	80	30	58	24	13	2	2	6
Ichthyophthirius sp.	.....	.....	.....	.....	.....	.....	.....	.....	on 3
Posthodiplostomum minimum (MacCallum)	In livers of 18, light	In livers of 78, light. In 3 hearts and 1 kidney	.....	.....	.....	.....	.....	.....	.....
Clinostomum sp.	.....	.....	In livers of 2	In livers of 9 and in mesenteries of 8	.....	In livers and mesenteries of 7	In mesenteries of 1	.....	In flesh of 1
Black grub (Strigeids)	.....	.....	.....	In flesh and skin of 13	.....	.....	.....	.....	.....
Proteocephalus ambloplites (Leidy)	1 ovary	.....	In livers of 3	In livers of 2	.....	.....	.....	.....	.....
Nematodes	17 nematodes in 1 body cavity	In 2 mesenteries	.....	.....	.....	45 in 1 stomach	.....	.....	.....
Leeches	1 on fin 1 on palate	.....	.....	.....	On fins of 3	.....	.....	.....	.....
Gill copepods	On 5	.....	.....	.....	.....	.....	.....	.....	.....
Argulus sp.	.....	.....	.....	.....	.....	.....	.....	.....	.....

bluegills with large fungus spots on their sides and fins was common and the mortality was fairly high. On May 24 a quarter-mile section of the shore line was cleared of dead fish and the following fish were taken:

1. Thirty-three dead bluegills ranging from 141 to 187 millimeters in length. All specimens had rotted, mycelia-coated sides and/or rotted, mycelia-coated fins.
2. Three large bluegills in a dying condition. The caudal fin of one fish was partly rotted off. The other two had diseased areas on their sides.
3. Three 250-millimeter crappies too badly decayed to be checked for *Saprolegnia* damage before death.
4. Three 100-millimeter bluegills without evidence of *Saprolegnia* infection.

On May 26 the shore line cleared of dead fish on May 24 was again inspected and the following fish were observed:

1. Thirty-five large, dead bluegills all showing the typical symptoms of rotted areas on the sides and fins.
2. Two large, live bluegills with tails rotted off.
3. Three small bluegills, dead but without symptoms.
4. One large, live warmouth with caudal and anal fins rotted off.

Observations indicated that the mortality from the epizootic reached a peak around May 28 and stopped completely around June 8.

A visit had been made to Red Haw May 13, 1947. At that time a number of dead bluegills were found along the shore line, and local residents reported a heavy die-off of bluegills a few weeks earlier. No conclusions as to the cause of the mortality were reached as a result of the 1947 observations, but it appears the mortality was the result of an epizootic similar to the one described above for the summer of 1948.

During the time that the Red Haw epizootic was at its peak, many observations were made at East Lake to learn if a similar die-off was occurring there. One large warmouth and two adult bluegills having the typical symptoms were found, but there were no further indications that the East Lake fish suffered from *Saprolegnia*. When nest building activity was at the peak, which was at the same time of the Red Haw epizootic, numerous dead adult bluegills were found near the nests in East Lake but none of these showed symptoms of *Saprolegnia* infection. It is likely that the periodic treating of the lake with copper sulphate prevented a *Saprolegnia* epizootic from developing in East Lake.

At the time of the Red Haw epizootic, East Lake bluegills were often found to have abrasions on their sides and fins. It was concluded that these abrasions were caused by the nest building activities, and that similar abrasions had made possible the Red Haw epizootic.

Black grubs, larval strigeids, were fairly abundant among the bluegills from both lakes (Table 2). The degree of infestation was higher in East Lake and again was thought to be correlated with the more frequent occurrence of the great blue heron on this lake.

The black grub of the yellow perch appeared to be a different

species than that which occurred among the bluegills. In the perch the grubs were invariably just under the skin whereas in the bluegills they were scattered through the body muscles.

Nematodes, gill copepods, leeches, *Argulus* sp., *Clinostomum* sp. and *Ichthyophthrius* sp. occurred in more or less insignificant numbers

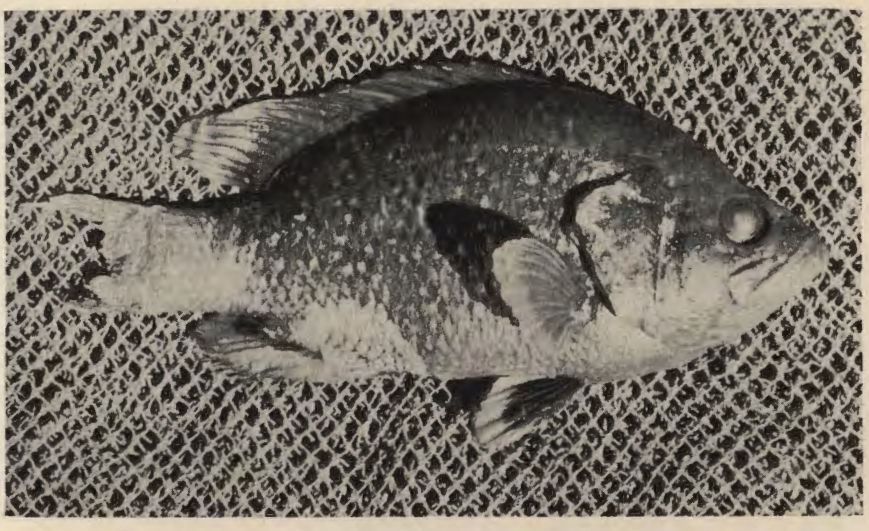

FIG. 2.—Warmouth with *Saprolegnia* infection. Red Haw Lake, June 1, 1949.

among one or more of the various fishes (Table 2). None of these parasites were thought to have been of any particular importance.

AGE AND GROWTH OF THE LARGEMOUTH BLACK BASS

In the management of artificial lakes for the production of fish populations to yield the maximum benefits to man, the largemouth black bass plays an important part. If a fish population is to be managed for the production of fish under natural conditions, it is necessary that a part of the population be made up of predatory fish capable of holding in check the increase in numbers of small fish. It is widely accepted that for most of the United States the largemouth is better suited to this purpose in artificial impoundments than any other species that has been tried. Not only is the largemouth outstanding as a predator, but also rates high as a game and food fish.

The body-scale relationship of both the Red Haw and East Lake bass was quite well defined by a straight line with an origin of 14.7 millimeters (Fig. 3). There was a slight suggestion of a sigmoid curve, but the deviation from the direct proportion was not enough to cause any appreciable error in the calculated lengths.

The coefficient of condition, K, of the bass from Red Haw is fairly high in comparison to reported values for bass from other areas (Table 3). The K for the specimens from East Lake is somewhat lower. The weight of the Red Haw bass increased approximately as the 3.075 power

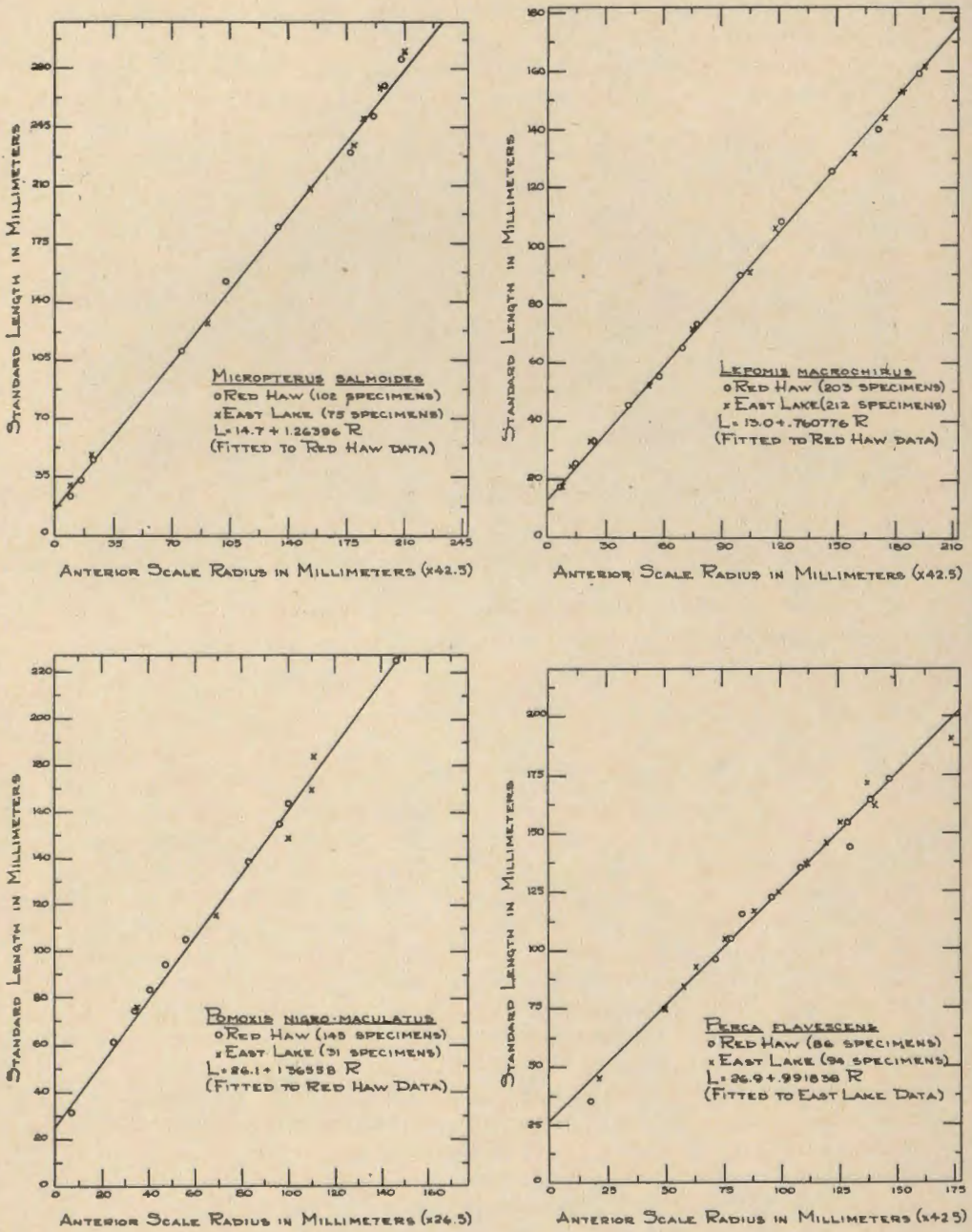

FIG. 3.—Body-scale relationships of largemouth black bass, bluegill, black crappie, and yellow perch from Red Haw and East Lakes.

TABLE 3

COEFFICIENTS OF CONDITION FOR LARGEMOUTH BLACK BASS FROM VARIOUS LOCALITIES

Location	Number Specimens	Range Standard Length mm.	Average K
Tennessee, Norris Res. (41)	774	53–484	2.48
Minnesota (14)	430	76–532	2.44
Minnesota (10)	156	.....	2.37
Iowa, Red Haw	62	96–291	2.35
Michigan (3)	1,100	46–392	2.29*
Iowa, East Lake	64	60–439	2.29
Iowa ponds (12)	33	38–251	2.25
Tennessee, Clinch R. (15)	118	200–299	2.19

* Beckman recorded K values ranging from 2.27 to 2.31, calculated from the length-weight relationship, based upon 1,100 specimens.

of the standard length, and that of the East Lake bass as the 3.058 power (Table 4).

Largemouth black bass fingerlings were rather difficult to obtain in both lakes. The mean lengths and range for the Red Haw collections, shown with the number of specimens in parenthesis, are:

June 28 ..... 25 mm. (19) ..... 18 to 30 mm.
July 15 ..... 31 mm. (18) ..... 26 to 40 mm.
July 23 ..... 36 mm. (6) ..... 31 to 40 mm.
Aug. 4 ..... 48 mm. (4) ..... 43 to 54 mm.
Aug. 22 ..... 44 mm. (1) .....

Similarly, those for East Lake are:

July 16 ..... 26 mm. (5) ..... 21 to 32 mm.
July 21 ..... 30 mm. (7) ..... 24 to 38 mm.
Aug. 3 ..... 43 mm. (3) ..... 38 to 49 mm.
Aug. 22 ..... 42 mm. (2) ..... 41 to 44 mm.
Sept. 1 ..... 65 mm. (1) .....

A comparison of the mean calculated lengths of the Red Haw and East Lake bass (Tables 5 and 6) indicates the East Lake bass grew faster than did those from Red Haw. The difference in growth was great enough to result in the East Lake fish reaching the legal minimum of ten inches total length in their third year, whereas the Red Haw fish did not attain this length until their fourth year. Tests of significance (Student's *t*) were made on the differences in the lengths at the end of the first five years of life. The differences for the first three years were highly significant, the probabilities being 0.0002 and below. The probabilities for the fourth and fifth year were 0.005 and 0.016 respectively.

TABLE 4

OBSERVED AND ESTIMATED WEIGHTS OF LARGEMOUTH BLACK BASS FROM RED HAW AND EAST LAKES

Standard Length, mm.	Number of Fish	Weight in Grams		
		Observed		Estimated*†
		Range	Mean	
Red Haw Lake				
96	2	15–22	18	20
106	2	24–27	26	27
124	2	41–50	46	44
149	1	.....	84	78
155	2	85–86	86	88
167	1	.....	117	111
173	1	.....	131	124
182	2	151–155	153	145
197	1	.....	187	181
212	4	218–255	219	226
229	1	.....	294	287
232	7	255–337	289	299
244	5	318–397	353	349
255	13	329–482	393	399
264	8	397–482	445	444
274	6	425–510	458	498
285	2	524–595	560	562
291	2	.....	610	601
East Lake				
65	1	.....	5	6
118	2	36–39	38	36
122	1	.....	39	41
134	2	57–58	58	53
143	1	.....	65	65
152	1	.....	86	79
192	2	169–196	182	165
206	2	210–215	212	199
217	3	227–238	232	233
226	4	241–264	256	264
237	4	280–350	322	305
245	6	308–381	345	338
254	10	350–482	399	377
263	7	397–458	426	419
273	8	425–483	464	470
282	2	520–524	522	519
294	5	510–624	544	590
300	1	.....	482	627
359	1	.....	1,134	1,086
387	1	.....	1,531	1,366
432	1	.....	1,701	1,913

* Red Haw: Log W = −4.789 + 3.075 Log L.
† East Lake: Log W = −4.777 + 3.058 Log L.

TABLE 5

CALCULATED AND MEASURED LENGTHS OF LARGEMOUTH BLACK BASS FROM RED HAW LAKE. SEXES COMBINED

Age Class	Number Specimens	Average Calculated Length at Each Annulus in mm.								Length at Capture
		1	2	3	4	5	6	7	8	
I	9	103								123
II	7	81	164							201
III	3	76	164	195						204
IV	19	73	140	198	244					254
V	13	70	131	189	234	260				265
VI	4	70	138	175	203	228	251			253
VII	1	62	94	140	173	198	232	262		267
VIII	1	91	168	239	335	388	419	451	464	464
Mean standard length mm.*		78	142	192	237	257	276	357	464	
Equiv. total length in.		3.7	6.8	9.2	11.4	12.3	13.2	17.1	22.2	
Annual increment		78	68	54	45	27	26	27	13	
Standard deviation		19.44	26.38	20.15	26.89	37.91				

* Fork length equals 1.168 standard lengths, based on 66 specimens 60 to 359 millimeters long. Total length equals 1.218 standard lengths based on 103 specimens 18 to 291 millimeters long.

TABLE 6

CALCULATED AND MEASURED LENGTHS OF LARGEMOUTH BLACK BASS FROM EAST LAKE. SEXES COMBINED

Age Class	Number Specimens	Average Calculated Length at Each Annulus in mm.										Length at Capture
		1	2	3	4	5	6	7	8	9	10	
I	7	113										142
II	4	86	205									218
III	14	89	175	233								251
IV	32	85	157	211	248							259
V	3	94	169	217	249	276						276
VII	1	78	160	200	262	289	312	340				359
VIII	2	106	166	251	310	350	376	396	425			412
X	1	135	178	221	271	301	335	357	399	415	432	432
Mean standard length mm.*		91	166	218	254	302	350	372	417	415	432	
Equiv. total length in.		4.4	7.9	10.4	12.1	14.4	16.7	17.8	19.9	19.8	20.7	
Annual increment		91	78	55	41	30	28	22	34	16	17	
Standard deviation		18.35	24.30	24.18	25.65	33.93						

* Fork length equals 1.162 standard lengths, based on 60 specimens 60 to 359 millimeters long. Total length equals 1.215 standard lengths, based on 82 specimens 10 to 439 millimeters long.

Two plausible explanations for the difference in growth of the two bass populations are as follows: (1) The greater abundance of golden shiner in East Lake may be important since this species is considered excellent bass food; (2) the continual greater transparency of the East Lake water might easily make food more accessible to the bass.

In length the 4-year-old bass from Red Haw and East Lake compare favorably with values reported from other localities (Table 7). It will be noted that they are almost identical with the values reported from most of the states of about equal latitude. A somewhat faster growth in the more southern latitude is to be expected.

TABLE 7

COMPARISON OF LENGTHS AT CAPTURE OF FOUR-YEAR-OLD LARGEMOUTH BLACK BASS FROM VARIOUS LOCALITIES

Locality	Number Specimens	Total Inches
Michigan, Third Sister Lake (7)	68	11.2
Michigan (4)	368	12.1
Iowa, Red Haw	19	12.2
Iowa, East Lake	32	12.4
Illinois, Sportsman Lake (43)	10	12.4
Wisconsin (5)	135	12.9
Wisconsin (26)	119	14.3
Minnesota (14)	319	14.4
Nevada, Lake Mead (32)	99	14.6
Tennessee, Norris Res. (41)	75	16.2
New Mexico, Conches (33)	54	18.1
Tennessee, Norris River (25)	109	20.8

Observations as to year class abundance were hampered by the fact that the principal method used for collecting bass, angling, is definitely selective as to size of fish taken. The fish below legal length were not sampled in proportion to their abundance. Therefore, the age classes III, II, and I (Tables 5 and 6) which are the year classes of 1945, 1946, and 1947, respectively, are not as strongly represented as they should be. It is justifiable to say that the 1943 and 1944 year classes in Red Haw and the 1944 and 1945 classes in East Lake were more abundant than were any older classes. The data are not sufficient to warrant further analysis, but it is of interest to point out that the number of bass stocked in Red Haw during 1943 was relatively low, and the number stocked in 1944 was relatively high. In East Lake the number stocked in 1944 was relatively high, whereas there were no bass stocked in 1945.

AGE AND GROWTH OF THE BLUEGILL

Just as the largemouth is the accepted predaceous species for stocking of artificial impoundments, the bluegill is the accepted forage species. It has a high reproductive potential, which means it is capable of furnishing large quantities of food for the bass and of maintaining itself

under heavy fishing pressure. However, with these desirable features and others, the status of the bluegill still warrants a great deal more investigation.

The calculations of body-scale relationship for the bluegills are based on the Red Haw collections. The observed values of the East Lake collections are plotted on the same graph (Fig. 3) to demonstrate the degree to which they agree with the Red Haw data. The limited amount of divergence of the points representing observed values from the calculated straight line strongly indicates a straight-line relationship for both lakes.

The length-weight equation for the Red Haw fish shows that their weight increased at a ratio slightly greater than the cube of the length,

TABLE 8

OBSERVED AND ESTIMATED WEIGHTS OF BLUEGILLS FROM RED HAW AND EAST LAKES

Standard Length, mm.	Number of Fish	Weight in Grams		
		Observed		
		Range	Mean	Estimated*†
Red Haw Lake				
36	2	2–3	2	2
45	5	3–5	3	3
56	12	5–11	7	7
65	23	6–13	10	11
74	17	11–19	15	16
84	5	16–27	22	24
94	10	27–41	34	33
104	13	36-66	48	46
112	1	.....	65	58
122	2	67–79	73	75
136	4	91–115	105	105
146	5	140–156	147	130
155	10	137–178	158	157
164	11	160–191	178	187
175	16	187–278	209	228
182	6	213–249	236	258
212	1	.....	454	413
East Lake				
56	7	4–10	8	7
66	3	11–13	12	12
75	7	16–22	18	17
84	5	18–21	20	24
94	16	23–41	32	33
103	14	31–56	44	44
116	2	55–66	60	62
125	4	64–95	78	77
136	11	92–107	100	98
144	27	100–133	120	116
150	27	124–166	139	131
162	15	138–186	159	163

* Red Haw: Log W = −4.572 + 3.090 Log L.
† East Lake: Log W = −4.238 + 2.920 Log L.

whereas in East Lake weight increased at a ratio slightly less than the cube (Table 8). The East Lake bluegills under eighty millimeters in length apparently were heavier for their length than those from Red Haw, however, not only did the larger bluegills from Red Haw show a greater weight than the East Lake fish, but the difference increased with increase in length.

The average coefficients of condition indicate that the fish in the two populations were of the same relative plumpness. In reality there was a difference between the two populations within the length range 140 to 170 millimeters. In this range, sixty-nine East Lake bluegills had an average K of 3.89, whereas the corresponding value for twenty-six specimens from Red Haw was 4.23. It is obvious that in the Red Haw the K value exhibits an upward trend with an increase in length up to 170 millimeters. By using a mean K value for all of the fish from each population the difference that exists over the 140 to 170 millimeter range has been obscured. The average values of K for the bluegills from both Red Haw and East Lake compare favorably with the values reported for this species in other localities (Table 9).

Five collections of young-of-the-year bluegills were obtained from Red Haw. The mean standard length and range of the fish in these collections are shown with the number of specimens given in parenthesis:

July 15	17 mm. (20)	15 to 19 mm.
July 23	17 mm. (13)	14 to 21 mm.
Aug. 4	20 mm. (15)	15 to 25 mm.
Aug. 22	22 mm. (19)	18 to 29 mm.
Oct. 23	28 mm. (15)	23 to 33 mm.

The measurements for East Lake fingerlings are:

July 16	19 mm. (15)	12 to 25 mm.
July 21	18 mm. (17)	12 to 25 mm.
Aug. 3	23 mm. (15)	16 to 31 mm.
Aug. 22	26 mm. (20)	19 to 37 mm.

Since bluegills are late spawners, the first year's growth is rather limited, and a considerable variation in the first year's growth can be expected. The average length of Red Haw fingerlings in October is similar to the calculated growth for the first year of life (Table 10).

The calculated growths (Tables 10 and 11) indicate that with the exception of the first and fourth years of life, the bluegills from Red Haw grew faster than did those from East Lake. The differences in annual increments are not great, but by the end of the fifth year of life the accumulated difference in favor of the Red Haw fish was thirteen millimeters. Inasmuch as the weight increases approximately as the cube of the length, only a small amount of difference in length results in a noticeable difference in weight.

Considering the fact that the growth of phytoplankton in East Lake

TABLE 9

COEFFICIENTS OF CONDITION FOR BLUEGILLS FROM VARIOUS LOCALITIES

Location	Number Specimens	Range Standard Length mm.	Average K
Indiana (19)	618	.....	3.17
Minnesota (10)	498	50–229	3.72
Michigan (3)	4,969	29–202	3.62*
Iowa, East Lake	138	50–169	3.94
Iowa, Red Haw	141	45–212	3.95

* Beckman recorded K values as ranging from 3.23 to 4.00, calculated from length-weight relationships based upon 4,969 specimens.

was suppressed by the use of copper sulphate, it is surprising that the difference in growth rate of the two bluegill populations was not greater than is indicated by the data. Fertilizing experiments (42) have shown the growth of bluegills to react to increases in phytoplankton. Were more older fish from both lakes available for analysis, the two growth rates might prove to differ more than is indicated by the limited data at hand.

The lengths at capture of the four- and five-year-old bluegills from both Red Haw and East Lake are about median in position when compared to the reported growths from other localities (Table 12).

The selectiveness of methods of collecting makes it difficult to determine year class abundance. Information on the young is best obtained

TABLE 10

CALCULATED AND MEASURED LENGTHS OF 133 BLUEGILLS FROM RED HAW LAKE. SEXES COMBINED

Age Class	Number Specimens	Average Calculated Length at Each Annulus in mm.								Length at Capture
		1	2	3	4	5	6	7	8	
I	43	30	.....	.....	.....	.....	.....	.....	.....	48
II	51	25	67	.....	.....	.....	.....	.....	.....	83
III	13	26	72	130	.....	.....	.....	.....	.....	141
IV	8	32	71	124	153	.....	.....	.....	.....	157
V	12	30	72	115	147	166	.....	.....	.....	170
VI	5	31	70	105	134	157	170	.....	.....	170
VIII	1	36	68	99	122	164	184	195	206	212
Mean standard length mm.*		28	69	123	145	163	172	195	206	.....
Equiv. total length in		1.4	3.4	6.1	7.2	8.1	8.6	9.7	10.3	.....
Annual increment		28	42	52	26	21	14	11	11	.....

* Fork length equals 1.191 standard lengths; based on 107 specimens 27 to 212 millimeters long. Total length equals 1.265 standard lengths; based on 142 specimens 19 to 212 millimeters long.

TABLE 11

CALCULATED AND MEASURED LENGTHS OF 145 BLUEGILLS FROM EAST LAKE. SEXES COMBINED

Age Class	Number Specimens	Average Calculated Length at Each Annulus in mm.						Length at Capture
		1	2	3	4	5	6	
I	25	32	.....	.....	.....	.....	.....	54
II	43	32	78	.....	.....	.....	.....	94
III	12	31	73	122	.....	.....	.....	136
IV	41	37	68	113	145	.....	.....	146
V	14	36	73	107	135	151	.....	156
VI	10	37	70	102	127	149	158	159
Mean standard length mm.*		34	73	112	140	150	158	.....
Equiv. total length in		1.7	3.6	5.6	7.0	7.5	7.9	.....
Annual increment		34	39	42	30	18	9	.....

* Fork length equals 1.204 standard lengths; based on 98 specimens 20 to 169 millimeters long. Total length equals 1.270 standard lengths based on 1.270 standard lengths; based on 144 specimens 10 to 169 millimeters long.

from field observations, whereas that for fish older than four years can be obtained only by angling. All year classes from 1942 on up to the 1948 class were well represented in Red Haw. In East Lake the 1944 year class appeared much more abundant than any other, but as in Red Haw the 1942 to 1948 year classes were represented. The 1943 year class was relatively more abundant in Red Haw. This is indicated by the following analysis of the year classes making up the collections from angling:

	Red Haw (32 specimens)	*East Lake* (52 specimens)
1946 year class	.....	2 per cent
1945 year class	31 per cent	12 per cent
1944 year class	19 per cent	50 per cent
1943 year class	31 per cent	17 per cent
1942 year class	19 per cent	19 per cent

The small difference in year class abundance was of importance since combined with the difference in growth rate and the difference in length-weight relationship, it resulted in a considerable difference in the size of the bluegills in the angler's catch from the two lakes. The following mean values of the lengths and corresponding estimated weights of bluegills taken by angling shows the magnitude of this difference:

Red Haw (40 specimens)	East Lake (60 specimens)
Mean length ..... 162 millimeters	Mean length ..... 151 millimeters
Estimated mean weight 126 grams	Estimated mean weight 106 grams

TABLE 1

COMPARISON OF LENGTHS AT CAPTURE OF FOUR-AND-FIVE-YEAR-OLD BLUEGILLS FROM VARIOUS LOCALITIES

Locality	Number Specimens	Total Inches
Four-Year-Olds		
Indiana, Springwood Lake (37)	113	4.2
Michigan, Deep Lake (8)	103	6.4
Michigan, Third Sister Lake (7)	72	6.5
Michigan (4)	1,774	6.6
Minnesota (14)	338	7.2
Iowa, East Lake	41	7.3
Ohio (27)	391	7.5
Illinois, Fork River (6)	21	7.5
Ohio (27)	329	7.6
Tennessee, Reelfoot (39)	292	7.7
Iowa, Red Haw	8	7.8
Indiana, northern part (36)	822	8.3
Indiana (19)	182	8.5
Indiana, Greenwood Lake (38)	54	8.7
Five-Year-Olds		
Michigan (4)	1,308	7.3
Michigan, Third Sister Lake (7)	63	7.4
Illinois (6)	5	7.6
Iowa, East Lake	14	7.8
Iowa, Red Haw	12	8.5
Minnesota (14)	127	8.5
Indiana, northern part (36)	313	9.1

AGE AND GROWTH OF THE BLACK CRAPPIES

The crappies do not exactly fit into the predator-prey relationship exemplified by the largemouth black bass and bluegill. In the artificial lakes of Iowa the crappies fill an ecological niche between the two extremes and probably make for the more efficient utilization of a lake's fertility. They compete with the bluegill for first place in support of the fishing in Iowa's artificial lakes.

Along with their good points the crappies have some objectionable ones. It appears that after a period of years the number of crappies in a lake tend to increase to the point at which they "take over" the lake and exist as a stunted population. One of the most urgent needs in the management of Iowa artificial lakes is to obtain an understanding of methods to prevent or remedy this overcrowding and stunting.

Black crappie were not very abundant in East Lake during the period of study and only a limited number of specimens could be obtained for study purposes. The calculated body-scale relationship (Fig. 3) for the species is based on the Red Haw specimens. The observed values for these fishes are accurately defined by the calculated straight line. There is a slight suggestion of a sigmoid curve but certainly not enough to cause any detectable error in growth calculations. The ob-

TABLE 13

OBSERVED AND ESTIMATED WEIGHTS OF BLACK CRAPPIES FROM RED HAW AND EAST LAKES

Standard Length, mm.	Number of Fish	Weight in Grams		
		Observed		Estimated*†
		Range	Mean	
Red Haw Lake				
56	3	3–4	4	5
64	9	6–9	7	7
78	12	8–17	11	11
85	14	13–20	17	17
95	13	15–30	24	24
106	17	28–43	36	34
116	12	46–61	51	45
123	4	53–70	61	54
136	3	65–86	76	73
146	8	88–103	98	91
155	15	79–134	114	110
164	9	119–164	138	130
171	3	136–171	151	148
182	1	.....	154	180
209	1	.....	269	275
214	3	294–305	298	296
226	4	309–355	337	350
234	4	343–411	376	390
245	1	.....	425	449
255	2	482–483	482	508
East Lake				
72	1	.....	13	13
80	2	16–17	16	17
103	1	.....	36	35
113	3	46–50	48	46
126	2	57–63	60	63
139	1	.....	80	83
146	2	98–103	100	96
154	2	99–125	113	112
165	5	125–160	140	136
173	10	139–169	157	156
186	3	166–207	184	192

* Red Haw Lake: Log W = −4.710 + 3.081 Log L.
† East Lake: Log W = −4.231 + 2.870 Log L.

served values for thirty-one East Lake black crappies are plotted on the same graph as the Red Haw values. Although the East Lake values show rather pronounced deviation from the straight line, it was necessary to use the body-scale relationship determined from the Red Haw data in the growth calculations for both populations.

The length-weight equations (Table 13) indicate that the weight of the Red Haw black crappies increased at a rate slightly greater than the cube of the length and that of the East Lake population increased

TABLE 14

COEFFICIENTS OF CONDITION FOR BLACK CRAPPIES FROM VARIOUS LOCALITIES

Location	Number Specimens	Range Standard Length Mm.	Average K
Indiana (19)	140	.....	2.47
Minnesota (10)	661	.....	2.75
Iowa, Red Haw Lake	133	30–279	2.93
Tennessee (41)	643	55–317	3.07
Iowa, East Lake	33	70–189	3.09
Minnesota, Lake of the Woods (11)	190	20–286	3.34

TABLE 15

CALCULATED AND MEASURED LENGTHS OF BLACK CRAPPIES FROM RED HAW LAKE. SEXES COMBINED

Age Class	Number Specimens	Average Calculated Length at Each Annulus in mm.								Length at Capture
		1	2	3	4	5	6	7	8	
I	85	61	.....	.....	.....	.....	.....	.....	.....	94
II	31	57	136	.....	.....	.....	.....	.....	.....	156
III	3	56	104	143	.....	.....	.....	.....	.....	153
IV	8	76	148	199	223	.....	.....	.....	.....	226
V	3	78	159	207	239	250	.....	.....	.....	250
VI	1	75	130	168	208	228	235	.....	.....	235
VIII	1	62	111	154	179	198	214	229	234	234
Mean standard length mm.*		61	137	186	222	235	222	229	234	.....
Equiv. total length in		3.1	6.9	9.4	11.2	11.9	11.2	11.6	11.8	.....
Annual increment		61	75	48	27	14	19	15	5	.....

* Fork length equals 1.218 standard lengths, based on 96 specimens 70 to 189 millimeters long. Total length equals 1.284 standard lengths, based on 132 specimens 30 to 279 millimeters long.

TABLE 16

CALCULATED AND MEASURED LENGTHS OF BLACK CRAPPIES FROM EAST LAKE. SEXES COMBINED

Age Class	Number Specimens	Average Calculated Length at Each Annulus in mm.				Length at Capture
		1	2	3	4	
I	7	56	.....	.....	.....	96
II	7	58	121	.....	.....	144
III	3	60	117	158	.....	166
IV	14	57	104	146	168	173
Mean standard length mm.*		57	110	148	168	.....
Equiv. total length in		2.9	5.6	7.4	8.4	.....
Annual increment		57	52	30	20	.....

* Fork length equals 1.203 standard lengths, based on 19 specimens 70 to 189 millimeters long. Total length equals 1.274 standard lengths based on 30 specimens 40 to 189 millimeters long.

at a rate slightly less than the cube. Notwithstanding this situation, the coefficient of condition values shows the East Lake fish within the length range collected to be heavier for a given length (Table 14). Assuming that the length-weight relationships continue on the same lines, the average K value for the East Lake population would eventually drop below that of Red Haw with an increase in the length of the specimens.

The average K value of the Red Haw black crappies is median in position when compared to those reported for black crappies from other areas. The value for East Lake is high.

Despite intensive sampling with a minnow seine, only one black crappie fingerling was taken from East Lake and thirteen from Red Haw. The mean lengths and ranges of the two Red Haw collections, number of specimens in parenthesis, were:

July 15	28 mm. (6)	24 to 31 mm.
Aug. 22	34 mm. (7)	24 to 38 mm.

The Red Haw black crappies grew much faster than those in East Lake (Tables 15 and 16). At the end of their fourth year the average calculated length of the East Lake specimens was 168 millimeters as compared with 22 millimeters for the Red Haw specimens. The length at capture of four-year-old fish shows a similar difference (Table 17). A catchable size crappie is about 170 millimeters. The East Lake fish reached this length during their fifth summer of life, whereas the Red Haw fish were of this size in their third summer.

The Red Haw four-year-olds showed a faster growth than any reported from other areas. The East Lake four-year-olds were among the slower growing groups (Table 17).

In East Lake the 1945, 1946, and 1947 year classes were about equally represented with the 1944 year class being more abundant. As has been noted, only one specimen of the 1948 year class was taken. In Red Haw the 1947 and 1946 year classes were strongly represented. No one of the older classes occurring in the collections (Table 15) appeared to be more strongly represented than any other.

TABLE 17

COMPARISON OF LENGTHS AT CAPTURE OF FOUR-YEAR-OLD BLACK CRAPPIES FROM VARIOUS LOCALITIES

Locality	Number Specimens	Total Inches
Illinois, Horseshoe Lake (43)	.....	7.8
Iowa, East Lake	14	8.7
Michigan (4)	.....	9.0
Tennessee, Reelfoot Lake (40)	109	9.6
Indiana (19)	63	10.0
Minnesota (14)	325	10.8
Iowa, Red Haw	8	11.4

AGE AND GROWTH OF THE WHITE CRAPPIE

E. B. Speaker, Superintendent of Biology Section, Iowa State Conservation Commission, who has investigated the fish populations of several different artificial impoundments of Iowa, is of the opinion that the white crappie is characteristic of badly silted and old "worn-out" ponds and lakes. Eschmeyer, Strand, and Jones (16, p. 92) made similar observations: ". . . in muddy TVA waters the white crappie predominates; in clear waters the black crappie may be expected to predominate." In this connection it is of interest to note that the white crappie was decidedly dominant in East Lake in 1948 although up to this date 18,500 black crappie had been stocked as compared to only 150 white crappies. In Red Haw the white crappie had been more heavily stocked, and yet the black was by far the more abundant.

Since the limited range of lengths of the white crappies from both East Lake and Red Haw prevented determination of the body-scale relationship, the calculated lengths were obtained on the assumption that the growth of the scale was directly proportional to that of the body.

Since the number of specimens involved in the Red Haw calculations is small and the length range covered by the East Lake specimens

TABLE 18

OBSERVED AND ESTIMATED WEIGHTS OF WHITE CRAPPIES FROM RED HAW AND EAST LAKES

Standard Length mm.	Number of Fish	Weight in Grams		
		Observed		
		Range	Mean	Estimated*†
Red Haw Lake				
88	2	.....	16	17
94	7	17–21	19	20
104	3	22–33	28	28
113	3	36–41	38	36
126	3	47–53	51	50
133	5	55–71	60	59
174	2	130–151	140	132
184	3	151–176	164	157
251	1	.....	366	403
East Lake				
94	3	11–44	24	22
154	2	99–131	111	96
167	16	105–131	115	122
174	44	105–158	132	138
183	14	133–186	153	160
196	4	189–199	192	196
203	1	.....	192	217
212	4	229–262	246	247
222	1	.....	272	283
252	1	.....	460	412

* Red Haw Lake: Log W = −4.675 + 3.034 Log L.
† East Lake: Log W = −4.484 + 3.675 Log L.

is limited, one is hardly justified in placing much confidence in the small amount of difference between the length-weight equations of the two populations (Table 18).

The K values of the specimens from the two populations are quite similar and no comment as to any difference is justified. The value for the Red Haw population based on twenty-nine specimens in the length range 88 to 251 millimeters is 2.50, and that for the East Lake fish based on ninety specimens in the length range 30 to 259 millimeters is 2.52. The only comparative data on K is from eighteen specimens from Minnesota (10) with an average K of 1.48.

The only white crappie fingerlings obtained from either lake were two 30-millimeter ones taken from East Lake on August 3. The Red Haw fish apparently grew much faster than those from East Lake (Table 19). The six-year-old white crappies from East Lake were considerably shorter than white crappies of the same age in Ohio (27) and in Reelfoot Lake, Tennessee (40).

The year class composition of the East Lake white crappies is most unusual. Trapping, creel censusing, and experimental angling all

TABLE 19

CALCULATED AND MEASURED LENGTHS OF WHITE CRAPPIES FROM RED HAW AND EAST LAKES. SEXES COMBINED

Age Class	Number Specimens	Average Calculated Length at Each Annulus in mm.							Length at Capture
		1	2	3	4	5	6	7	
Red Haw Lake									
I	21	63							110
II	5	50	155						173
III	1	58	132	170					170
VI	1	99	143	162	196	222	238		251
Mean standard length mm.*		62	150	166	196	222	238		
Equiv. total length in.		3.1	7.6	8.4	9.9	11.2	12.1		
Annual increment		62	92	28	34	26	16		
East Lake									
I	1	34							84
II	2	36	100						100
III	1	25	77	147					165
IV	1	29	78	142	178				183
V	6	42	106	130	155	168			169
VI	73	42	104	131	150	166	180		180
VII	1	45	115	152	168	185	203	212	212
Mean standard length mm.†		4	104	132	151	166	180	212	
Equiv. total length in.		2.1	5.3	6.7	7.7	8.4	9.1	10.7	
Annual increment		41	63	27	20	16	14	9	

* Fork length equals 1.228 standard lengths; based on 15 specimens 88 to 251 millimeters long. Total length equals 1.288 standard lengths; based on 29 specimens 88 to 251 millimeters long.

† Fork length equals 1.209 standard lengths based on 60 specimens 30 to 229 millimeters long. Total length equals 1.285 standard lengths based on 85 specimens 30 to 229 millimeters long.

strongly indicated that the 1942 year class, on a numerical basis, made up more than 80 per cent of the population. This dominance of an older year class plus the slow growth rate indicates that these fish had become overcrowded and as a result were stunted.

There was a second rather unusual feature of the East Lake white crappies. Out of eighty-one over 160 millimeters long, only 15 were males. No indication of sexual segregation was noted. The males that were taken were obtained by all the different sampling methods. Furthermore the sampling methods were the same as those used in Red Haw where no such unequal abundance of sexes was found. It therefore, appears that the sex ratio of the white crappie population of East Lake was out of balance. Thompson and Bennett (43) observed a similar condition in white crappies from Horseshoe Lake, Illinois. They attributed the condition to the males being shorter-lived than the females.

TABLE 20

OBSERVED AND ESTIMATED WEIGHTS OF YELLOW PERCH FROM RED HAW AND EAST LAKES.

Standard Length mm.	Number of Fish	Weight in Grams		
		Observed		
		Range	Mean	Estimated*†
Red Haw Lake				
96	3	16–21	18	19
104	8	22–30	27	24
115	4	25–38	30	32
123	6	32–45	39	39
135	9	42–55	47	51
144	8	57–67	63	61
154	22	62–86	73	75
164	20	71–112	91	90
173	3	99–114	106	105
216	1	.....	205	201
East Lake				
44	1	.....	2	2
79	2	8–9	8	11
83	2	11–14	12	13
93	3	13–18	16	17
104	6	18–26	22	23
116	12	25–37	32	31
125	21	32–53	40	39
136	7	37–55	48	49
146	4	48–66	60	59
155	18	59–104	71	70
164	15	73–120	86	82
172	5	91–122	100	93
186	1	.....	126	115
196	1	.....	126	133
205	1	.....	168	151

* Red Haw Lake: Log W = −4.530 + 2.928 Log L.
† East Lake: Log W = −6.954 + 2.753 Log L.

AGE AND GROWTH OF THE YELLOW PERCH

The yellow perch is not usually considered a suitable species for use in ponds and smaller artificial lakes. It appears that in this type of environment it fails to reach a desirable size, and such was the case with the Red Haw and East Lake perch. It was desirable to know whether this small size was the result of excessively slow growth or whether the fish were too young to be of any size.

The rate of increase in weight of the yellow perch from both lakes was less than the cube of the length (Table 20), but that for the Red Haw specimens was above that of the East Lake fish.

The average K value for the yellow perch from Red Haw was 2.05 and that for the East Lake specimens 1.96. In comparison to K values of the yellow perch from sixteen other areas, the values of the Red Haw and East Lake specimens are median in position (Table 21).

No young-of-the-year yellow perch were obtained from Red Haw. From East Lake three taken on June 30 had an average length of forty-four millimeters, one taken on July 6 was forty-four millimeters, and one taken August 3 was sixty-four millimeters.

The body-scale relationship of the yellow perch from both lakes was satisfactorily described by a straight line (Fig. 3). The calculated lengths indicate the yellow perch of Red Haw and East Lake to have grown the same amount their first year of life (Table 22). In the second year the Red Haw specimens grew much faster. The growth rates of both populations are sufficient to produce catchable-size fish in a reasonable length of time. It appears that the preponderance of small fish making up the yellow perch populations was due to year class abundance rather than to slowness of growth. As the methods of collecting appeared effective for all size perch except fingerlings, it is considered that the different year classes are fairly accurately represented in the collections.

TABLE 21

COEFFICIENTS OF CONDITION FOR YELLOW PERCH FROM VARIOUS LOCALITIES

Location	Number Specimens	Range Standard Length mm.	Average K
Indiana (19)	723	.....	1.57
Michigan (3)	.....	43–291	1.78*
Michigan, Saginaw Bay (21)	818	.....	1.79
Wisconsin, Green Bay (22)	938	.....	1.87
Michigan, Lake Erie (21)	.....	.....	1.91
Minnesota (10)	2,774	.....	1.95
Iowa, East Lake	99	40–209	1.96
Iowa, Red Haw	84	90–219	2.05
Wisconsin, Lake Mendota (18)	95	.....	2.09
Michigan, Lake Michigan (22)	294	.....	2.18

* Beckman recorded K values as ranging from 1.69 to 1.86, calculated from the length-weight relationship based upon 4,274 specimens.

Of the eighty-seven specimens from Red Haw all except one of the fish were of the 1947 and 1946 year classes. Of the eighty-eight specimens from East Lake all except two specimens were from the 1944 to 1947 year classes.

According to local fishermen small perch have been characteristic of both Red Haw and East Lake for several years. We can at least assume that the fish are not young only because they have recently become established in the lakes. They are known to have been in these lakes for several years. Yellow perch populations made up primarily of small fish are characteristic of certain lakes. On the basis of their own observations and a review of the literature up to the time, Adams and Hankinson (1, p. 427) concluded:

> In some lakes all perch are small, regardless of size and depth or other apparent conditions, while in others they run large . . . A fully satisfactory explanation for this diversity of size in different bodies of water has not been made.

The writer is of the opinion that a predominance of small perch (6 to 8 inches, total length) is characteristic of lakes which do not have strata of water with sufficient oxygen that is of a temperature suitable

TABLE 22

CALCULATED AND MEASURED LENGTHS OF 87 YELLOW PERCH FROM RED HAW AND EAST LAKES. SEXES COMBINED

Age Class	Number Specimens	Average Calculated Length at Each Annulus in mm.						Length at Capture
		1	2	3	4	5	6	
Red Haw Lake								
I	21	80						112
II	65	76	152					153
III	1	74	168	216				216
Mean standard length mm.*		77	152	216				
Equiv. total length in.		3.7	7.2	10.3				
Annual increment		77	76	48				
East Lake								
I	16	72						99
II	31	73	119					124
III	28	70	123	154				151
IV	11	69	116	143	161			162
V	1	75	110	134	154	164		164
VI	1	63	108	142	166	184	196	196
Mean standard length mm.†		71	120	150	161	174	196	
Equiv. total length in.		3.4	5.7	7.1	7.6	8.2	9.3	
Annual increment		71	49	30	19	14	12	

* Fork length equals 1.143 standard lengths; based on 83 specimens 30 to 219 millimeters long. Total length equals 1.201 standard lengths; based on 83 specimens 30 to 219 millimeters long.

† Fork length equals 1.147 standard lengths; based on 92 specimens 40 to 209 millimeters long. Total length equals 1.208 standard lengths based on 101 specimens 40 to 209 millimeters long.

for the larger perch. In lakes such as Red Haw and East Lake, during the summer the deeper water (approximately 10 feet or deeper) normally inhabited by larger perch has an extremely low oxygen content, whereas the more shallow water frequented by the smaller perch usually is not depleted of oxygen. This theory is dependent upon a difference in habitat requirement between the large and small perch, and there is strong evidence that such a condition does exist. Adams and Hankinson (1) and Reighard (35) definitely concluded that larger perch favor the deeper water, whereas the smaller ones favor the more shallow water. Pearse and Achtenberg (34) working primarily with larger fish, concluded that the yellow perch is an inhabitant of the deeper waters. The difference in distribution between the smaller and larger fish is probably due to temperature requirements. It therefore appears that a lake may provide an excellent environment for smaller perch but a very poor or completely unsuitable environment for the larger ones.

A comparison of length at capture of two-year-old yellow perch with data reported from other localities indicates the Red Haw specimens had a relatively high growth rate, whereas those from East Lake had a relatively low growth rate (Table 23).

AGE AND GROWTH OF THE WESTERN GOLDEN SHINER

The golden shiner is often considered by many a suitable forage species to be used with the largemouth black bass. The golden shiner was scarce in Red Haw but fairly abundant in East Lake. The information given here is based entirely on specimens from the latter lake.

The length-weight relationship formula (Table 24) shows the specimens to have increased in weight at a rate somewhat greater than the cube of the length. The coefficient of condition of sixty-five shiners, 110 to 169 millimeters long, was 2.12.

The data were not sufficient for the calculation of the body-scale relationship, therefore, the direct proportion relationship, a straight line from zero, was used.

TABLE 23

LENGTHS AT CAPTURE OF TWO-YEAR-OLD YELLOW PERCH FROM VARIOUS LOCALITIES

Locality	Number Specimens	Total Inches
New York, Mohawk & Hudson Watershed (17)	80	5.6
Michigan (4)	1,576	5.8
Massachusetts (31)	71	5.9
Iowa, East Lake	31	5.9
Minnesota, Lake of the Woods (9)	184	6.1
Indiana (19)	240	6.1
Minnesota (14)	109	6.3
Wisconsin, Green Bay (22)	58	6.5
Indiana, Muskellunge Lake (38)	98	7.0
Iowa, Red Haw	65	7.2
Wisconsin, Lake Mendota (18)	29	7.8

TABLE 24

OBSERVED AND ESTIMATED WEIGHTS OF GOLDEN SHINERS FROM EAST LAKE

Standard Length mm.	Number of Fish	Weight in Grams		
		Observed		
		Range	Mean	Estimated*
119	1	.....	35	34
124	3	35–40	37	39
133	11	38–62	48	49
144	24	48–74	63	63
154	17	66–100	83	79
165	9	91–111	98	99

* Log W = −5.306 + 3.294 Log SL.

The East Lake golden shiners (Table 25) appear to have grown rapidly in comparison to the rates reported by Cooper (13) for New York and Michigan waters. The length at capture of 33 East Lake four-year-old specimens was 414 millimeters as compared to 92.2 millimeters for 146 specimens from Michigan waters and 95.2 millimeters for 20 specimens from New York waters.

Some indication of the growth of young-of-the-year golden shiners may be obtained from the periodic collections made by use of a minnow seine. The mean lengths and ranges for the collections, with the number of specimens in parenthesis, are:

June 30 ..... 18 mm. (9) ..... 13 to 20 mm.
July 16 ..... 25 mm. (13) ..... 19 to 29 mm.
July 21 ..... 26 mm. (4) ..... 21 to 28 mm.
Aug. 3 ..... 20 mm. (1) .....

CHANNEL CATFISH AND BULLHEADS

The channel catfish is generally not considered as a species suitable for use in the stocking of lakes. The primary objection to it for this purpose is that it usually does not spawn in the lake environment and thereby fails to maintain itself. Since the species has not been widely stocked in lakes and seldom occurs in them naturally, there is practically no knowledge as to the growth and welfare of this fish in lakes. With the increase in fishing pressure, intensive management of lakes is becoming more popular. A species of fish can hardly be totally disregarded simply because it fails to reproduce in the environment to be managed. In fact the failure to reproduce may prove to be a desirable characteristic of fish used for stocking lakes. If a species does not reproduce in a lake, man is then directly in control of the numbers of fish added each year and therefore circumvents the problems of overcrowding due to natural reproduction. The fact that a species does not

TABLE 25

CALCULATED AND MEASURED LENGTHS OF 60 GOLDEN SHINERS FROM EAST LAKE. SEXES COMBINED.

Age Class	Number Specimens	Average Calculated Length at Each Annulus in mm.					Length at Capture
		1	2	3	4	5	
I	6	56	.....	.....	.....	.....	56
II	1	58	122	.....	.....	.....	122
III	33	27	85	137	.....	.....	141
IV	15	37	95	137	154	.....	154
V	5	39	82	117	140	155	158
Mean standard length mm.*		36	88	135	150	155	.....
Equiv. total length in.		1.8	4.3	6.7	7.4	7.7	.....
Annual increment		36	54	48	18	15	.....

* Fork length equals 1.138 standard lengths; based on 69 specimens 40 to 169 millimeters long. Total length equals 1.255 standard lengths; based on 97 specimens 10 to 169 millimeters long.

reproduce does not mean that it must be stocked every year. In East Lake fishing for channel catfish is reported to have been fairly good since 1935. During the summer of 1948 on basis of creel census work it was estimated that a hundred two-to-eighteen pound specimens of these fish were taken, and yet the last reported stocking was 1,172 yearlings stocked in 1940, and all the reported stocking previous to 1940 amounted to only 3,010 fish. If such a rate of return as has been realized in East Lake can be expected from other lake stockings, the periodic stocking of channel catfish is justifiable. There are, of course, areas where the channel catfish is not in demand, but in the artificial lake region of southern Iowa it is held in high esteem as both a sport and a pan fish.

The little life history data obtained on the channel catfish came, with one exception, from fish taken by anglers. This method of sampling yields incomplete information since most fishermen are not too pleased at the prospect of having their fish cut open for detailed examinations. With the exception of one dead specimen, no channel catfish were taken in Red Haw. The information that follows pertains to specimens taken from East Lake.

Inasmuch as the channel catfish does fail to reproduce normally in some lakes, it is of value to consider the condition of the sex organs of the specimens taken from East Lake. With the exception of one 450-millimeter specimen taken June 2 and a 485-millimeter specimen taken April 17, all of the specimens obtained were taken in August and September. The channel catfish in Iowa is reported to spawn from June 1 to June 20 (2). The April 17 and June 2 specimens were females, and the ovaries of both contained only a few poorly developed eggs.

The ovaries of females taken later in the season were similar in appearance. A fisherman reported taking a female which contained a double handful of well developed eggs.

The average coefficient of condition for eleven specimens, 348 to 746 millimeters, was 1.86 (Table 26).

Information on the age and growth of channel catfish is very limited. In the present work the number of vertebral rings is given for a few specimens (Table 26). It is not claimed that these counts represent an accurate estimate of age, but there is, perhaps, some correlation between the ring count and age of the fish. Indications of such a correlation have been demonstrated by Lewis (28) for the northern black bullhead and by Hooper (23) for the tadpole madtom, *Schilbeodes mollis* (Hermann). Emphasis was placed upon obtaining bullheads from the two lakes, but even then only seventeen were obtained from Red Haw and none were obtained from East Lake. Therefore the important result of the work is the indication that bullheads were scarce in both lakes although they had been heavily stocked at least in Red Haw. It is admitted that the sampling method may not have taken bullheads in proportion to their abundance, but the methods including angling were certainly effective enough to have detected a sizable population had it been present.

In addition to indicating a small adult population, the work also showed bullhead reproduction in the lakes to be extremely low. As is commonly known, young bullheads swarm at the surface over an extended period and are easily observed, if at all abundant in a lake or pond. Even though the lakes were observed more or less daily throughout the summer, no such swarms were seen. One swarm was observed

TABLE 26

DATA ON CHANNEL CATFISH FROM EAST LAKE, 1948

Standard* Length mm.	Weight in Ounces	Sex	Number of Vertebral Rings	Data Collected
348	25			
	32		2	Aug. 11
450	49			
457	56	F	6	Aug. 2
485	82	F	7	Apr. 17
510	98			
595	149			
601	152			
605	140			
	160	M	11	Sept. 14
648	195	M	8	Aug. 8
	213	M	8	Aug. 8
730	234	M	8	Sept. 12
756	296	F	13	Aug. 2

* Fork length equals 1.088 standard lengths, and total length equals 1.190 standard lengths, based upon eleven fish.

by the concessionist at Red Haw. Intensive use of a minnow seine in various habitats yielded one five-millimeter bullhead from Red Haw on June 28.

The gonads of the adult bullheads taken from Red Haw appeared normal, and there is no reason to believe the fish failed to spawn. The answer to the low population probably is the loss of the young fish to predators. Inasmuch as so few young-of-the-year fish were observed, the loss is indicated to have occurred during the fry stage. Work on farm ponds indicates that bullheads cannot maintain themselves in small ponds in which an established largemouth black bass population is present.

A limited amount of data was obtained from thirteen northern black bullheads, two northern yellow bullheads, and two northern brown bullheads all from Red Haw (Table 27). The length conversion factor for standard and total length for the thirteen northern blacks which ranged from 134 to 321 millimeters in length was 1.173. The average coefficient of condition for these same fish was 2.34. The numbers

TABLE 27

DATA ON BULLHEADS FROM RED HAW LAKE, 1948

Standard Length mm.	Total Length in Inches	Weight in Grams	Sex	Date Collected	Number of Vertebral Rings
134	6.2	54	F	May 25	3
141	6.5	59	.....	.....	.....
147	6.8	65	F	May 25	3
169	7.8	102	F	April 10	3*
175	8.1	139	.....	.....	.....
181	8.4	156	.....	.....	.....
190	8.8	176	F	May 14	3
200	9.2	190	M	June 8	3
202	9.3	195	M	May 26	4
219	10.1	250	.....	.....	.....
232	10.7	342	.....	.....	.....
254	11.7	369	F	Sept. 3	4
321	14.8	680	M	Sept. 3	6
		Northern Yellow Bullhead			
155	7.1	83	M	Sept. 3	1
289	13.3	638	M	Sept. 3	6
		Northern Brown Bullhead			
186	8.5	146	F	May 3	1*
212	9.7	202	F	May 3	3

* The ring for past winter was thought not to be completed at the time the fish were collected.

of vertebral rings for the bullheads are given in Table 27. But as indicators of age they must be accepted with caution as was noted in regard to the channel catfish data.

SUMMARY

Two eighty-acre lakes, Red Haw and East Lake, were studied from March 26 to September 22, 1948. These lakes are located in the same valley near Chariton, Iowa.

The fish populations were sampled by use of a minnow seine, a bag net, hoop nets, basket traps, gill nets, and angling. The species most abundant in both lakes were: largemouth black bass, bluegill, yellow perch, black crappie, and white crappie. In addition warmouth were quite abundant in Red Haw, and channel catfish and golden shiner were fairly abundant in East Lake.

The fish specimens collected were weighed, measured, and examined for parasites, and scale samples were taken for age and growth studies. The rate of growth, parasitic infestation, and coefficient of condition along with some general information were determined and summarized for the individual species from each lake. With this information, comparisons were made between the Red Haw and East Lake populations and between these populations and populations which have been studied in other areas.

Three important parasitic infections were found among the fishes of the two lakes. The plerocercoid stage of *Proteocephalus ambloplitis* occurred in large numbers in the ovaries of thirty of the forty female largemouth black bass examined. Metacercariae of *Posthodiplostomum minimum* heavily infested the livers of 255 of the 256 bluegills examined. In addition, this parasite also infested the heart and kidney of all the East Lake bluegills and more than half of the Red Haw bluegills. The bluegills of East Lake were much more heavily parasitized than were the Red Haw fish. This difference in degree of parasitism was attributed to the more frequent occurrence of the definitive host, the great blue heron, on East Lake. An epizootic of *Saprolegnia* sp. of approximately two weeks' duration occurred in Red Haw during late May and early June. This epizootic was primarily among the larger bluegills and caused a very noticeable mortality. Continuous observations failed to show any similar epizootic in East Lake, probably due to the copper sulphate treatments. In addition to the three more important species of parasites, the following forms also occurred: larval Strigeids, *Clinostomum* sp., leeches, *Argulus* sp., and parasitic copepods of the gills.

A comparison of growth rates showed that the largemouth black bass from East Lake had grown more rapidly than those from Red Haw and that the bluegills, black crappies, white crappies, and yellow perch from Red Haw had grown more rapidly than those from East Lake. The differences in growth rates between the fishes of Red Haw and East Lake were not as great as was anticipated on basis of the

suppression of phytoplankton growth in East Lake by the use of copper sulphate.

With the exception of four individuals, all the yellow perch taken from the two lakes were less than 175 millimeters long. It was suggested that this small average size was due to an absence of water with sufficient oxygen and of a temperature suitable for the larger perch.

Although positive age determinations could not be made on the channel catfish, the dates of stocking, the number of fish stocked compared to reported yield, and observed yield of excellent specimens in 1948 indicated that this species has a high rate of survival and grows well in East Lake. There was, however, no evidence of natural reproduction.

The length-at-capture of Red Haw and East Lake fishes of specified ages compared with similar reports from other areas is as follows:

1. The largemouth black bass from both lakes; East Lake bluegills, black crappies, and yellow perch; and Red Haw warmouths were about average.
2. The Red Haw bluegills, black crappies, and yellow perch were above average.
3. The East Lake white crappies were below average.

Age class composition of the various species in the two lakes was somewhat obscured by the selectiveness of the sampling methods, but, nevertheless, a few definite conclusions could be reached.

1. The largemouth black bass population of both lakes was made up primarily of fish 6 years old or younger.
2. The white crappie population of East Lake was composed largely of 6-year-old females.
3. The bluegill population of East Lake was dominated by 4-year-old fish.
4. The yellow perch population of Red Haw was made up almost entirely of 2-year-old fish whereas that of East Lake included many more 3-year-olds.
5. Yearling black crappies were extremely abundant in Red Haw.
6. In the Red Haw warmouth population all ages below 6 or 7 years appeared to be more or less well represented.

LITERATURE CITED

1. Adams, C. C. and T. L. Hankinson
1928. The ecology and economics of Oneida Lake fish. Bull. of the New York State Coll. of Forestry, Syracuse Univ. Roosevelt Wildlife Annals 1:235–548.

2. Aitken, W. W.
1936. Some common Iowa fishes. Iowa Agr. Exp. Sta., Circ. 224.

3. Beckman, William C.
1948. The length-weight relationship factors for conversions between standard and total lengths, and coefficients of condition for seven Michigan fishes. Trans. Amer. Fish. Soc. 1945. 75:237–56.

4. ———
1949. The rate of growth and sex ratio for seven Michigian fishes. Trans. Amer. Fish. Soc. 1946. 76:63–81.

5. BENNETT, GEORGE W.
1937. The growth of the largemouth black bass, *Huro salmoides* (Lacepede), in the waters of Wisconsin. Copeia 1937:104–188.

6. ———, D. H. THOMPSON AND SAM A. PARR
1940. Lake management reports. 4. A second year of fisheries investigations at Fork Lake, 1939. Illinois Nat. Hist. Survey, Biol. Notes No. 14.

7. BROWN, C. J. D. AND ROBERT C. BALL
1943. A fish population study of Third Sister Lake. Trans. Amer. Fish. Soc. 1942. 72:177–86.

8. CARBINE, W. F. AND VERNON C. APPLEGATE
1948. The fish population of Deep Lake, Michigan. Trans. Amer. Fish. Soc. 1945. 75:200–27.

9. CARLANDER, KENNETH D.
1942. An investigation of Lake of the Woods, Minnesota, with particular reference to the commercial fisheries. Minnesota Dept. of Cons., Bur. of Fish. Res. Invest. Rept. No. 42. (Typewritten).

10. ———
1944. Notes on the coefficient of condition, K, of Minnesota fishes. Minnesota Dept. of Cons., Bur. of Fish. Res. Invest. Rept. No. 41. (Revised, typewritten).

11. ———
1944. Notes on the minor species of fish taken in the commercial fisheries at Lake of the Woods, 1939 to 1943. Minnesota Dept. of Cons., Bur. of Fish. Res. Suppl. 2 of Invest. Rept. No. 42. (Typewritten).

12. ——— AND REYNOLD A. FREDIN
1948. Project No. 37. Management of small ponds for fish production. Quarterly Rept. of the Iowa Coop. Wildlife Res. Unit., Oct., Nov., and Dec. 1948:101–105.

13. COOPER, GERALD P.
1936. Age and growth of the golden shiner (*Notemigonus crysoleucos auratus*) and its suitability for propagation. Mich. Acad. Sci., Arts, and Letters. 21:587–97.

14. EDDY, SAMUEL AND KENNETH D. CARLANDER
1942. Growth rates studies of Minnesota fish. Minnesota Dept. of Cons. Bur. of Fish. Res. Invest. Rept. No. 28. (Mimeo).

15. ESCHMEYER, R. W.
1944. Fish migration into the Clinch River below Norris Dam, Tennessee. Jour. Tennessee Acad. Sci. 19:31–41.

16. ———, R. H. STROUD AND A. D. JONES
1944. Studies of the fish population on the shoal area of a TVA main-stream reservoir. Jour. Tennessee Acad. Sci., 19:70–122.

17. GREELEY, J. R.
1934. Fishes of the Raquette Watershed with annotated list. pp. 53–108 in a biological survey of the Raquette. Supp. to the 23rd Annual Rept. New York Cons. Dept. for 1933.

18. HASLER, ARTHUR D.
1945. Observations on the winter perch populations of Lake Mendota. Ecology 26:90–94.

19. HILE, RALPH
1931. Rate of growth of fishes of Indiana. Invest. of Indiana Lakes No. 2 Dept. Cons. Div. Fish and Game, Indiana Publ. No. 107. pp. 9–55.

20. ———
1936. Age and growth of the Cisco, *Leucichthys artedi* (Le Sueur), in the lakes of the northeastern highlands, Wisconsin. Bull. U. S. Bur. Fish. 43:211–317.

21. ——— AND FRANK W. JOBES
1941. Age, growth, and production of the yellow perch, *Perca flavescens* (Mitchill) of Saginaw Bay. Trans. Amer. Fish. Soc. 1940. 70:102–22.

22. ——— AND ———
1942. Age and growth of the yellow perch, *Perca flavescens* (Mitchill) in the Wisconsin waters of Green Bay and northern Lake Michigan. Papers Michigan Acad. Sci., Arts and Letters. 1941. 27:241–66.

23. HOOPER, FRANK F.
1949. Age analysis of a population of the Ameiurid fish, *Schillbeodes mollis* (Hermann). Copeia 1949:34–38.

24. HUNTER, G. W., III
1942. Studies on the parasites of fresh-water fishes of Connecticut. Section IV in A Fishery Survey of Important Connecticut Lakes. State of Connecticut Public Doc. No. 47. State Board of Fish. and Game. Lake and Pond Survey Unit Bull. No. 63. pp. 228–88.

25. JONES, ALDEN M.
1941. The length of the growing season of largemouth black bass in Norris Reservoir, Tennessee. Trans. Amer. Fish. Soc. 1940. 70:183–87.

26. JUDAY, CHANCEY AND CLARENCE L. SCHLOEMER
1938. Growth of game fish in Wisconsin waters—fifth report. Notes from Limnological Laboratory of Wisconsin Geol. and Nat. His. Survey, Apr. 1938. (Mimeo).

27. LANGLOIS, T. H.
Ohio's fish program. Ohio Div. Cons. and Nat. Resources. (not dated).

28. LEWIS, W. M.
1949. The use of vertebrae as indicators of the age of the northern black bullhead *Ameiurus m. melas* (Rafinesque). Iowa State Coll. Jour. of Sci. 23:209–18.

29. ———
1949. Fisheries investigations on two artificial lakes in southern Iowa. I. Limnology and vegetation. Iowa State Coll. Jour. of Sci. 23(4):355–361.

30. ——— AND T. S. ENGLISH
1949. The warmouth, *Chaemobryttus cornarius* (Bartram), in Red Haw Hill Reservoir, Iowa. Iowa State Coll. Jour. of Sci. 23(4):317–322.

31. MCCABE, BRITTON C.
1942. Fishes. Section 3 in Fish. Survey Rept. 1942. Mass. Dept. of Cons.

32. MOFFETT, JAMES W.
1943. A preliminary report on the fishery of Lake Mead. Trans. North Amer. Wildlife Conf. :179–86.

33. MOTTLEY, C. M. AND T. K CHAMBERLAIN
1948. Management of game fish of Conchos Reservoir. Prog. Fish. Cult. 10:177–86.

34. PEARSE, A. S. AND H. ACHTENBERG
1920. Habits of yellow perch in Wisconsin Lakes. Bull. U. S. Bur. Fish. 36:297–366.

35. REIGHARD, J. E.
1915. An ecological reconnaissance of the fishes of Doublas Lake, Cheboygan County, Michigan, in midsummer. Bull. U. S. Bur. Fish. 33:215-49.

36. RICKER, WILLIAM E.
1942. The rate of growth of bluegill sunfish in lakes of Northern Indiana. Invest. of Indiana Lakes and Streams 2:161–214.

37. ———
1942. Fish populations of two artificial lakes. Invest. of Indiana Lakes and Streams 2:256–65.

38. ———
1945. Natural mortality among Indiana bluegill sunfish. Ecology 26:111–21.

39. SCHOFFMAN, ROBERT J.
1938. Age and growth of the bluegills and largemouth black bass in Reelfoot Lake. Jour. Tennessee Acad. Sci. 13:81–103.

40. ———
1940. Age and growth of the black and white crappie, the warmouth bass, and the yellow bass in Reelfoot Lake. Jour. Tennessee Acad. Sci. 15:22–42.

41. STROUD, RICHARD H.
1948. Growth of the basses and black crappie in Norris Reservoir, Tennessee. Jour. Tennessee Acad. Sci. 23:31–99.

42. SWINGLE, H. S. AND E. V. SMITH
1939. Increasing fish production in ponds. Trans. of the 4th North Amer. Wildlife Conf. 1939:332–38.

43. THOMPSON, DAVID H. AND GEORGE W. BENNETT
1938. Lake management reports. 1. Horseshoe Lake near Cairo, Illinois. Illinois Nat. His. Survey Biol. Notes No. 8.

44. ——— AND ———
1939. Lake management reports. 3. Lincoln Lakes near Lincoln, Illinois. Illinois Nat. His. Survey Biol. Notes. No. 11.

ON THE SPECIFICITY OF THE SPARING PHENOMENON *

ELERY R. BECKER, THOMAS M. SCHWINK, DORWIN BYRD, AND REX CONN.

From the Department of Zoology and Entomology
Iowa State College

Received January 12, 1950

It was recently reported by Becker, Brodine, Marousek, and Byrd (1) that the plasma of ducks which have survived the primary attack of *Plasmodium lophurae* possesses the property of protecting parasitized duck erythrocytes against removal from the circulating blood of chicks. To produce this phenomenon the duck plasma is injected intravenously into the young chick's circulation an hour or two before the parasitized duck cells.

Normal duck plasma possesses a similar property, though its effect may not be so pronounced. The property of plasma producing this effect has been called its "sparing action." The desirability of further studies on the sparing phenomenon is strongly indicated. The most timely of these studies would concern the specific nature of the phenomenon, i.e., whether the observed effects are attributable to extrinsic factors or to a more or less peculiar property of ducks' blood.

MATERIALS AND METHODS

The ducks, chicks, and strain of parasite were the same as in the previous experiments reported by Becker, et al. (1). The procedures were also similar with the few exceptions to be noted. In order to save time, it was made a practice to read only 30 microscopic fields of 100 erythrocytes when no parasites were observed instead of continuing to 100 fields. The chicks in which the tests were made were in general a little younger than before, although it is not yet certain that this change resulted in any improvement in getting more clear-cut results. Another rule that was observed was the elapse of ninety minutes to two hours between the plasma injection into the chick and the subsequent inoculation with the parasitized duck erythrocytes. The bloods to be tested were drawn from recovered ducks one to several days before the probable date selected for inoculating the chicks, the plasma was separated immediately by centrifugation, and afterwards frozen and stored in a refrigerator. In certain instances to be noted subsequently the plasma was taken from either normal or actively infected ducks.

Certain minor changes were made in the washing of the infected

*This investigation was supported (in part) by Research Grant 605 (C) from the Division of Research Grants and Fellowships of the National Institute of Health, United States Public Health Service.

duck erythrocytes which probably contributed more than any other to improvement of our technique. Sixteen cc. of blood was drawn from the right jugular vein of a duck with 70 per cent to 90 per cent parasitized erythrocytes and usually starting the sixth, sometimes the seventh, day of its infection. It was made standard practice to use ducks one-half to two-thirds grown for donors of parasitized cells. Eight cc. of the blood was transferred from the syringe into each of two sterile 15 cc. screw-cap test tubes containing the appropriate amount of dried heparin. The tubes were rotated gently end-for-end in the vertical plane for a minute, filled with warm (40°C.) sterile 0.85 per cent salt solution, rotated again to mix, and centrifugalized at about 2,000 r.p.m. for 5 or 6 minutes. The supernatant was drawn off with suction, the tubes again filled with warm (40°C.) physiological salt solution, rotated to mix, and centrifugalized. Two more washings followed these first, and after the last or fourth, the washed erythrocytes were suspended in the correct amount of the warm (40°C.) salt solution to make the concentration desired. The arithmetical calculation of the density of the suspension was, of course, based on the per cent of parasitized cells read from stains smears of the donor duck and the number of erythrocytes per cubic centimeter of blood obtained by hemocytometer reading, both made just before the blood was drawn.

The data obtained from reading the stained blood smears of the test chicks are recorded by series in Table 1, each series in turn consisting of groups variously treated. The statistical constants appearing

TABLE 1

MEAN PER CENTS OF PARASITIZED CELLS AND STANDARD DEVIATIONS ± FOR GROUPS OF CHICKS SUBJECTED TO TREATMENTS AND CONTROL GROUPS

(Explanation: The notations after the series designations refer to (1) the age of the chicks at the time of inoculation; (2) the number of parasitized duck erythrocytes injected per 100 g. of each chick's body weight; (3) the volume of test materials injected per 100 g. of each chick's body weight.)

(Abbreviations: i.d.p. = immune duck plasma; i.d.s. = immune duck serum; n.d.p. = normal duck plasma; i.c.p. = immune chick plasma; n.c.p. = normal chick plasma; inf.d.p. = plasma from infected duck at peak of primary attack; + = < 0.05%.)

SERIES 1

(Age, 16 days; 5.5 x 10^8 P. C.; Gs 1–4, 0.5 cc. day 0, 0.8 cc. days 1 and 2)

Group	No. Chicks	Time After Inoculation					
		5 min.	1 day	2 days	3 days	5 days	7 days
1 ..... (saline)	6	1.46 0.22	2.44 1.46	2.89 1.85	16.0 11.6	45.7 19.6	17.7 4.6
2 ..... (i.d.p.)	6	1.83 0.30	5.76 4.74	5.41 5.38	21.3 15.6	17.3 8.9	1.0 1.1
3 ..... (saline)	6	1.44 0.28	1.22 1.30	1.07 1.03	8.24 7.25	23.5 19.6	11.8 8.0
4 ..... (i.d.p.)	6	1.32 0.62	3.03 3.39	3.53 3.20	15.03 13.03	9.7 6.8	0.17 0.18

SERIES 2

(Gs 1 and 3, age 14 days; Gs 2 and 4, age 21 days; 5.5 x 10^8 P. C.; Gs 1–4, 1.0 cc day 0)

Group	No. Chicks	Time After Inoculation: 5 min.	10 hrs.	1 day	2 days	4 days
1	6	3.37	4.43	6.75	12.00	53.0*
(saline)		0.81	4.10	6.27	8.14	25.3
2	6	2.75	5.90	9.23	13.50	46.5*
(saline)		1.24	4.15	7.05	9.51	30.8
3	6	3.47	9.47	16.48	27.50	73.4*
(n.d.p.)		0.47	1.33	4.36	6.75	5.5
4	6	3.57	8.08	15.30	25.08	76.2*
(n.d.p.)		0.52	1.73	4.05	5.66	7.3

* 5 chicks surviving.

SERIES 3

(Gs 1 and 2, age 19 days; Gs 3 and 4, age 12 days; 5.5 x 10^8 P. C.; 1.0 cc. day 0)

Group	No. Chicks	Time After Inoculation: 3 min.	1 hr.	6 hrs.	12 hrs.	1 day	2 days	3 days	4 days	5 days	6 days	8 days
1	6	3.7	2.7	1.6	1.4	1.9	3.2	17.0	26.9	40.9	40.0	17
(saline)		0.5	0.5	0.8	0.7	2.1	3.8	17.2	17.3	30.7	20.2	18
2	6	3.2	3.0	2.1	2.2	4.5	5.3	22.7	21.9	15.6	2.9	2
(i.d.p.)		0.4	0.3	0.9	1.3	4.1	5.1	20.4	17.5	15.0	2.5	2
3	6	2.8	1.0	0.4	0.2	0.3	0.4	2.5	4.1	8.0	17.2	41
(saline)		0.3	0.7	0.4	0.3	0.5	0.5	4.3	4.1	10.3	11.2	25
4	6	3.4	2.5	2.1	1.6	4.6	5.4	22.8	25.1	28.7	5.1	2
(i.d.p.)		0.6	0.4	0.3	0.6	4.0	4.1	12.5	9.8	11.8	4.8	3

SERIES 4

(Age, 11 days; 5 x 10^8 P. C.; 1 cc. day 0)

Group	No. Chicks	Time After Inoculation: 3 min.	4.5 hrs.	20 hrs.	44 hrs.	3 days	5 days	7 days	10 days	12 days
1	10	1.43	0.05	0.07	0.21	0.50	3.1	17.8	40.5	14
(saline)		0.93	0.04	0.04	0.14	0.36	2.0	8.4	20.2	17
2	10	1.32	0.04	0.10	0.29	0.57	4.1	15.0	24.5	31
(saline-heparin)		0.78	0.05	0.08	0.31	0.37	3.6	9.9	15.0	14
3	10	2.87	2.17	5.73	8.16	30.98	14.4	1.5	0.4	+
(i.d.p.)		0.57	0.75	3.07	4.03	13.60	12.7	2.8	1.3	+

SERIES 5

(Age, 14 days; 6 x 10^8 P. C.; G 1, 0.6 cc. day 0; Gs 2 and 4, 0.9 cc. day 0; G 3, 0.9 cc. i.d.p. and 0.6 cc. i.c.p. day 0)

Group	No. Chicks	Time After Inoculation							
		3 min.	4.5 hrs.	1 day	3 days	5 days	7 days	8 days	10 days
1 ..... (i.d.p.)	7	3.1 0.7	4.0 1.0	10.2 4.7	37.0 9.8	24.2 8.7	0.5 0.6	0.3 0.6	0.0 0.0
2 ..... (i.c.p.)	7	3.2 0.4	2.5 0.9	5.6 4.7	20.4 12.6	22.7 9.3	0.7 0.7	0.1 0.3	0.0 0.0
3 ..... (i.d.p., i.c.p.)	7	3.7 0.5	2.9 0.5	7.2 3.4	35.3 13.9	19.1 14.7	0.2 0.3	0.1 +	0.0 0.0
4 ..... (saline)	8	3.1 0.2	0.10 0.05	0.2 0.3	1.3 0.7	9.9 4.8	31.3 18.9	18.2 17.4	2.4 3.1

SERIES 6

(Age, 11 days; 5 x 10^8 P. C.; Gs 1–3, 0.5 cc. day 0)

Group	No. Chicks	Time After Inoculation										
		3 min.	1 hr.	5 hrs.	1 day	3 days	4 days	5 days	6 days	7 days	9 days	12 days
1 ..... (i.d.p.)	9	2.8 0.2	2.3 1.2	2.3 0.9	2.0 1.0	11.9 6.8	19.1 9.8	18.3 10.4	6.6 6.9	2.8 4.4	1.7 3.2	0.1 +
2 ..... (saline-heparin)	5	2.5 0.4	0.3 0.2	0.1 0.2	0.1 0.1	1.1 0.8	5.8 4.4	6.7 4.7	26.5 18.0	25.2 15.0	30.7 14.4	23.9 18.4
3 ..... (rat plasma)	5	1.5 0.7	0.2 0.2	0.1 0.2	0.1 0.1	0.6 0.4	2.0 1.5	4.6 4.6	14.5 8.0	20.5 10.4	40.1 14.4	27.2 15.7

SERIES 7

(Age, 9 days; 7 x 10^8 P. C.; 0.6 cc. day 0 and 0.8 cc. day 1)

Group	No. Chicks	Time After Inoculation										
		3 min.	1 hr.	5 hrs.	20 hrs.	3 days	4 days	5 days	6 days	7 days	9 days	12 days
1 ..... (n.d.p., 5-day-old ducks)	6	2.5 0.6	1.7 0.4	0.7 0.8	1.2 1.9	8.7 13.9	16.5 22.6	23.5 16.2	36.9 13.4	23.5 9.0	17.6 10.7	4.5 9.0
2 ..... (n.d.p., 10-day-old ducks)	6	3.0 1.0	2.2 0.5	1.4 0.6	1.5 1.0	10.2 7.0	16.9 6.1	34.5 18.1	43.4 5.7	32.2 11.0	11.1 9.7	6.7 8.1
3 ..... (i.d.p., old ducks)	6	2.4 0.5	2.7 0.5	2.4 0.8	3.0 1.4	20.1 12.0	30.3 14.6	30.6 10.7	10.7 10.4	5.9 8.8	0.4 0.8	+ +
4 ..... (saline)	6	1.7 0.6	0.2 0.2	0 0	+ +	0.1 +	0.3 0.2	1.3 0.8	7.0 3.5	8.5 6.2	33.0 15.6	23.8 17.3

SERIES 8

(Age, 12 days; 4 x 10^8 P. C.; Gs 1–4, 0.8 cc. days 0 and 1)

Group	No. Chicks	Time After Inoculation: 3 min.	1 hr.	5 hrs.	1 day	2 days	4 days	5 days	6 days	7 days	9 days	13 days
1 ..... (chicken egg albumen)	10	2.0 **0.60**	1.10 **1.2**	0.66 **0.71**	1.0 **0.86**	4.1 **3.5**	23 **16**	25 **17**	38 **33**	30 **18**	28 **19**	14 † **21**
2 ..... (i.d.p.)	10	1.6 **1.6**	1.30 **0.36**	1.50 **0.75**	2.9 **1.3**	11.6 **4.2**	34 **9**	23 **8**	6.6 **6.8**	2 **4.9**	0.5 **0.9**	1.3 **3.9**
3 ..... (saline)	5	1.6 **0.36**	0.68 **0.41**	0.86 **0.49**	1.6 **1.1**	5.4 **3.5**	31 **15**	37 **20**	59 **6.4**	47 **19**	46* **14**	3.3‡ **2.1**
4 ..... (no saline)	5	1.9 **0.4**	0.88 **0.52**	0.80 **0.67**	1.4 **1.4**	5.4 **6.2**	25 **16**	29 **20**	54 **18**	33 **12**	23 **14**	8.4* **14**

* 4 chicks surviving; † 8 chicks surviving; ‡ 3 chicks surviving.

SERIES 9

(Age, 10 days; 5 x 10^8 P. C.; Gs 1–5, 0.5 cc. day 0)

Group	No. Chicks	Time After Inoculation: 1 min.	5 hrs.	16 hrs.	2 days	4 days	6 days	8 days	11 days	14 days
1 ..... (more resistant chick p.)	12	1.8 **0.6**	0.4 **0.6**	0.3 **0.1**	2.2 **0.3**	8.1 **4.0**	35 **20**	15 **18**	4.1 **9.5**	4.8 **12.2**
2 ..... (less resistant chick p.)	11	1.2 **0.5**	0.1 **0.1**	0.2 +	0.6 **0.1**	1.4 **0.6**	11 **9**	16 **13**	1.6 **2.0**	0.4 **1.0**
3 ..... (saline)	6	2.3 **1.1**	1.3 **1.5**	1.2 **0.6**	5.3 **2.1**	13.5 **2.8**	51 **19**	20 **11**	1.3 **0.6**	0 **0**
4 ..... (i.d.p.)	4	3.4 **1.0**	4.7 **1.1**	4.4 **1.3**	16.8 **5.5**	43.1 **14.7**	11 **6**	0.9 **1.1**	0 **0**	0 **0**
5 ..... (2-day-old duckling plasma)	8	2.8 **0.3**	3.4 **1.0**	3.5 **1.6**	14.2 **4.2**	35.4 **15.1**	60 **11**	(7) 15 **11**	5.3 **4.9**	2.0 **4.1**

SERIES 10

(Age, 9 days; 2 x 10^8 P. C.; Gs 1–5, 0.5 cc. day 0, and 0.7 cc. day 2)

Group	No. Chicks	Time After Inoculation: 3 min.	1 hr.	5 hrs.	22 hrs.	4 days	7 days	9 days
1 ..... (i.d.p.)	8	0.88 **0.42**	0.57 **0.33**	0.40 **0.39**	0.25 **0.32**	2.5 **1.7**	17.0 **13.3**	9.9 **9.1**
2 ..... (i.d.s.)	8	1.11 **0.44**	0.64 **0.29**	0.41 **0.20**	0.17 **0.63**	2.1 **1.5**	9.7 **7.5**	5.2 **4.7**
3 ..... (saline)	8	0.37 **0.21**	0 **0**	0 **0**	0 **0**	0.1 **0.6**	1.4 **0.6**	7.1 **5.1**
4 ..... (human p.)	6	0.20 **0.16**	0 **0**	+ +	+ +	0.1 **0.1**	1.0 **0.4**	8.5 **3.1**
5 ..... (duck egg albumen)	8	0.74 **0.11**	0.12 **0.17**	+ +	+ +	0.1 **0.1**	4.1 **3.2**	17.6 **16.0**

SERIES 11

(Age, 11 days; 5×10^8 P. C.; Gs 1–3, 0.5 cc. days 0 and 1)

Group	No. Chicks	Time After Inoculation								
		2 min.	4 hrs.	1 day	3 days	4 days	5 days	6 days	8 days	10 days
1	6	2.1	1.2	3.3	17.8	20.3	29.7	13.0	6.8	6.1
(i.d.p. D. #15)		**0.36**	**0.62**	**2.5**	**13.3**	**12.6**	**19.2**	**8.5**	**7.1**	**12.5**
2	6	2.5	1.4	5.1	25.6	30.0	47.6	30.4	14.0	8.5
(i.d.p. D. #29)		**0.32**	**0.26**	**4.0**	**18.2**	**14.4**	**18.3**	**9.9**	**16.7**	**7.8**
3	6	2.4	0.6	1.1	6.5	9.4	14.8	19.0	12.8	14.5
(saline)		**0.36**	**0.74**	**1.3**	**9.5**	**12.4**	**21.4**	**23.2**	**7.2**	**18.6**

SERIES 12

(Age, 9 days; 2×10^8 P. C.; Gs 1 and 2, 0.5 cc. day 0; Gs 4 and 5, 0.5 cc. day 0 and 1, and 0.7 cc. day 2)

Group	No. Chicks	Time After Inoculation							
		2 days	4 days	5 days	6 days	7 days	9 days	11 days	13 days
1	7	0.40	10.0	19	40	24	6.5	1.3	2.1
(n.d.p.)		**0.14**	**6.0**	**11**	**17**	**9**	**5.0**	**1.7**	**4.9**
2	7	0.40	9.7	19	46	33	22.6	11.8	8.7
(inf.d.p.)		**0.28**	**2.4**	**12**	**14**	**13**	**13.1**	**16.6**	**14.0**
3	8	0.04	1.2	3	14	9	19.8	25.0	16.8
(control)		**0.06**	**2.0**	**3**	**14**	**6**	**12.4**	**20.1**	**18.1**
4	8	1.10	12.0	21	30	17	9.7	11.6	7.1
(n.d.p.)		**1.01**	**8.3**	**13**	**11**	**9**	**9.0**	**12.4**	**10.2**
5	8	1.20	14.1	26	44	35	25.2	13.1	2.2
(inf.d.p.)		**1.12**	**11.4**	**15**	**20**	**13**	**13.4**	**10.9**	**2.8**

SERIES 13

(Age, 9 days; 4×10^8 P. C.; Gs 1–4, 0.8 cc. day 0 and 0.6 cc. day 1)

Group	No. Chicks	Time After Inoculation								
		20 min.	1 day	2 days	4 days	5 days	6 days	7 days	10 days	12 days
1	6	2.1	2.4	7.4	36	33	23	10.3	0.5	0
(i.d.p. D. #15)		**0.57**	**1.0**	**2.47**	**8.1**	**17**	**18**	**8.9**	**0.6**	**0**
2	6	1.8	1.5	6.3	31	28	27	3.7	+	0
(i.d.p. D. #29)		**0.80**	**0.56**	**2.2**	**14**	**8.4**	**15**	**4.4**	+	**0**
3	6	0.17	0.05	0.15	0.9	2.2	8.9	14.9	33	18.2
(saline)		**0.22**	**0.07**	**0.16**	**0.45**	**1.0**	**4.8**	**6.3**	**20**	**19.3**
4	6	1.8	1.8	4.0	37	34.1	38	24	11	10.7
(i.d.p. D. #30)		**0.66**	**1.6**	**1.8**	**16**	**12.0**	**14**	**9.5**	**6.7**	**13.9**

SERIES 14

(Age, 12 days; 4 x 10^8 P. C.; Gs 1, 2 and 3, 0.5 cc. days 0)

Group	No. Chicks	Time After Inoculation							
		20 min.	1 day	2 days	4 days	5 days	6 days	8 days	10 days
1	7	1.1	2.9	6.1	32	33	34	14.9	20.0
(saline)		0.6	3.5	4.8	21	22	19	9.7	20
2	7	2.0	3.5	9.6	35	28	24	9.4	1.2
(i.d.s.)		0.2	2.4	4.8	11	18	19	10	2.1
3	7	1.5	3.3	6.2	23	33	43	29.1	13.9
(guinea egg albumen)		0.7	3.3	4.6	17	25	27	6.2	13.2

SERIES 15

(Age, 8 days; 3.2 x 10^8 P. C.; Gs 1 and 2, 0.6 cc. day 0; Gs 3 and 4, 0.6 cc. days 0, 1 and 3)

Group	No. Chicks	Time After Inoculation						
		50 min.	22 hrs.	2 days	4 days	5 days	6 days	8 days
1	5	3.0	3.4	10.7	36	21	6.1	0.3
(i.d.p.)		0.5	1.5	6.2	8	7	5.0	0.3
2	6	1.6	1.8	4.8	30	44	36	22.0
(saline)		1.1	1.3	4.1	24	21	22	18.9
3	5	3.2	4.3	11.5	38	28	11.9	2.5
(i.d.p.)		0.2	0.7	3.9	7	19	12.1	3.0
4	5	1.5	1.7	3.4	28	25	35.4	25.0
(guinea pig p.)		1.1	1.2	2.9	18	18	22.1	21.4

SERIES 16

(Age, 9 days; 4.5 x 10^8 P. C.; Gs 1 and 4, 0.3 cc.; Gs 2, 3 and 5, 0.6 cc.;

Gs 6 and 7, 1.6 cc.; all injections on day 0)

Group	No. Chicks	Time After Inoculation										
		45 min	1 day	2 days	4 days	5 days	6 days	7 days	9 days	11 days	13 days	16 days
1	5	2.0	4.8	5.3	31	38	14	7.4	2.0	0.04	0	0
(guinea p.)		0.4	0.7	0.7	12	13	9.1	8.8	2.7	0.05	0	0
2	5	1.9	8.3	10.0	46	34	15	7.8	1.7	0.14	0	0
(guinea p.)		0.3	3.1	3.5	10	10	12	9.3	2.5	0.28	0	0
3	6	1.3	2.9	3.3	23	27	29	19.4	15.6	21	22.9	14.2
(saline)		0.8	4.0	4.4	28	31	24	15.0	9.5	22	23.6	8.7
4	5	1.3	3.1	5.7	32	20	8.3	4.7	1.1	0.05	0	0
(guinea p.)		1.1	2.9	6.4	27	14	12	6.0	2.1	0.31	0	0
5	5	2.0	3.9	5.3	40	25	4.7	1.1	+	0	0	0
(guinea p.)		1.0	2.2	2.3	16	11	3.3	1.4	+	0	0	0
6	2	1.7	6.0	6.6	39	28	2.5	1.5	+	0	0	0
(guinea p.)		0.1	0.6	2.4	1	5	1.3	1.2	+	0	0	0
7	3	1.8	5.3	8.9	60	54	40	21.1	18.3	14.0	1.7	+
(i.d.p.)		0.3	2.2	2.9	15	17	25	12	15.9	14	0.7	+

SERIES 17

(Age, 10 days; 2 x 10^8 P. C.; G 1, no injection; G 2, 0.5 cc. days 0 and 1)

Group	No. Chicks	Time After Inoculation						
		1 day	2 days	4 days	5 days	6 days	7 days	10 days
1 ..... (control)	8	1.0 0.3	2.5 2.1	36 11	48 19	33 17	16 10	5.9 7.1
2 ..... (n. turkey p.)	6	1.2 0.4	4.5 2.0	28 9	38 12	30 14	12 9	0.6 1.1

SERIES 18

(Age, 11 days; 2.5 x 10^8 P. C.; Gs 1–3, 0.4 cc. day 0; G 4, 0.3 cc. day 0)

Group	No. Chicks	Time After Inoculation								
		2 min.	1 day	3 days	4 days	5 days	6 days	7 days	9 days	11 days
1 ..... (guinea egg albumen)	5	0.84 0.33	0.46 .54	3.0 3.3	9.4 12.6	13.1 14.5	24 23	20 19	22 17	6 5
2 ..... (guinea pig p.)	5	0.64 0.20	0.35 .22	1.8 1.4	8.2 6.6	9.9 6.1	31 16	32 13	35 19	22 23
3 ..... (saline)	4	0.67 0.17	0.68 0.67	6.7 6.7	14.1 20.4	21.1 19.9	28 17	24 13	33 18	31 29
4 ..... (inf.d.p.)	5	0.96 0.28	0.82 .43	6.1 5.1	14.2 10.8	23.4 16.5	44 22	35 11	32 14	18 19

SERIES 19

(Age, 12 days; 3.75 x 10^8 P. C.; Gs 1–4, 0.6 cc. on day 0)

Group	No. Chicks	Time After Inoculation								
		2 min.	3 hrs.	1 day	3 days	5 days	7 days	9 days	11 days	13 days
1 ..... (i.c.p.)	7	1.9 0.5	0.4 0.2	0.7 0.3	4.4 2.4	25 12	44 12	34 12	24 8	14 11
2 ..... (n.c.p.)	7	1.2 0.6	0.1 +	0.1 0.1	0.9 0.5	5.5 2.3	10 9	5 8	0.2 0.3	0.1 0.2
3 ..... (n.c.p.)	7	2.0 0.2	1.0 0.7	1.2 1.2	7.4 7.2	23 10	20 13	18 20	14 20	12 21
4 ..... (saline)	8	1.9 0.6	2.2 0.3	3.4 0.6	18.1 7.7	56 10	37 12	27* 15	32* 24	18* 17

* 3 chicks surviving.

SERIES 20

(Age, 11 days; 4.0 x 10^8 P. C.; Gs 1–3, 0.8 cc. day 0; Gs 4 and 5, 0.8 cc. days 0, 1, 2, and 3)

Group	No. Chicks	Time After Inoculation								
		3 min.	5 hrs.	1 day	2 days	4 days	5 days	6 days	8 days	10 days
1	4	2.88	2.5	3.2	10.8	37	19	1.4	0	0
(i. guinea p.)		0.51	0.5	1.5	5.4	12	7	1.0	0	0
2	4	2.73	3.5	3.9	10.2	53	60	25	3.9	3.8
(n. guinea p.)		0.41	0.3	1.2	2.6	8.4	8	5	3.7	4.8
3	8	1.78	1.3	1.2	2.8	24	33	37	29	26.3
(saline)		0.78	1.0	1.0	2.6	18	27	14	15	17.4
4	4	2.28	2.7	3.3	10.3	55	55	26	8.0	9.5
(n. guinea p.)		0.20	0.3	1.0	1.3	7.1	13	12	8.9	9.3
5	4	2.68	2.6	3.7	14.8	34	21	6.8	1.8	0
(i. guinea p.)		0.46	0.6	0.8	5.5	10	23	9.5	3.0	0

SERIES 21

(Age, 11 days; 4 x 10^8 P. C.; Gs 1 and 2, 0.6 cc.)

Group	No. Chicks	Time After Inoculation									
		1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	10 days	12 days
1	5	0.1	0.6	0.4	1.8	2.3	14	15	41	51	30
(control)		0.1	0.8	0.1	0.4	0.6	5	5	14	16	13
2	3	0.3	1.3	2.3	11	15	25	18	41	43	24
(guinea egg albumen)		0.3	1.2	2.9	13	17	11	6	8	12	6

SERIES 22

(Age, 11 days; 5 x 10^8 P. C.; G 1, 0.8 cc.; G 2, 0.4 cc.; G 3, uninjected)

Group	No. Chicks	Time After Inoculation											
		3 min.	1 hr.	5.5 hr.	1 day	2 days	3 days	4 days	5 days	6 days	8 days	10 days	14 days
1	10	2.90	2.53	3.15	6.3	10.0	29	44	24	3.2	0.32	+	0
(n. goose p.)		0.53	0.96	1.00	3.7	4.1	12	11	5	3.8	0.75	+	0
2	10		2.49	3.65	8.9	15.6	40	48	27	7.1	0.93	0	0
(n. goose p.)			0.51	0.77	3.8	3.6	3	8	11	6.4	2.46	0	0
3	10		1.98	1.84	4.8	7.5	22	33	20	8.5	1.60	+	0
(control)			1.06	1.12	3.9	5.9	15	18	13	5.6	2.60	+	0

SERIES 23

(Age, 12 days; 3 x 10^8 P. C.; G 1, 0.4 cc. day 0; G 2, 0.8 cc. day 0; G 3, no injection.)

Group	No. Chicks	Time After Inoculation									
		45 min.	2 days	3 days	4 days	5 days	6 days	7 days	9 days	11 days	13 days
1 ..... (turkey serum)	11	1.21 **0.27**	1.62 **1.31**	6.10 **4.85**	9.2 **6.2**	16.4 **12.4**	7.3 **10.0**	4.8 **11.0**	0.43 **1.19**	+ +	0 **0**
2 ..... (turkey serum)	10	1.08 **0.13**	1.89 **0.93**	6.80 **3.20**	11.2 **7.4**	25.9 **17.9**	20.4 **15.8**	17.0 **19.5**	1.85 **4.30**	0.16 **0.37**	+ +
3 ..... (control)	11	0.38 **0.38**	0.20 **0.30**	0.93 **1.47**	1.5 **2.4**	4.2 **6.7**	9.1 **12.1**	7.5 **8.5**	8.50 **9.20**	14.0 **21.2**	10.2 **16.8**

in the table under the heading "Time after inoculation" are the means of the per cents of parasitized cells for the groups after various intervals of time and, below them in bold-face type, the standard deviations. The formula for the standard deviation was, as before, the square root of the following quantity: the sum of the squared variables divided by the actual number of chicks, minus the square of the mean. It is to be clearly understood that the data apply to readings of the per cent of parasitized erythrocytes in stained smears of the blood of young chicks which were inoculated with washed parasitized duck erythrocytes, and that the chicks had been injected with "immune" duck plasma, salt solution, or other test liquids two hours or less before inoculation with the parasitized erythrocytes. The volume of plasma, serum, etc. injected is recorded in the table in terms of cubic centimeters per 100 g. of chick body weight.

As in the previous investigation (1), the Fisher small-sample method was used in some instances to test the significance of differences between groups while in a few other cases analysis of variance was indicated. It is realized that the statistical validity of these tests depends on the normality of the distribution of the variables, and that it is not known for sure if the distribution of parasite counts made in the blood of chicks infected with *P. lophurae* is normal at any or every interval after inoculation, but it is felt that the tests nevertheless have a definite usefulness.

RESULTS

The results are to be presented topically instead of by series of experiments. Each heading suggests a hypothetical objection to the validity of the specific nature of the sparing phenomenon that must be tested. Frequent references are made to groups of the various series constituting Table 1 in support of the argument.

THE REALITY OF THE SPARING EFFECT

While it is believed by the writers that the previous publication proved the sparing effect, results with the procedure incorporating the minor improvements noted above were even more clear-cut. Even so,

the magnitude of the differences between test and control birds was exceedingly inconstant, as is revealed in the following summary of the ratios of the mean parasitized cell counts, on selected days, for "immune" duck plasma receptors to the mean counts for the related control groups:

(*Series*)	(*Groups Compared*)	(*Time After Inoculation*)	(*Ratio*)
1	2 vs. 1	1 da.	5.76:2.44 = 2.36
1	4 vs. 3	1 da.	3.03:1.22 = 2.48
2	3 vs. 1	1 da.	16.48:6.75 = 2.44
2	4 vs. 2	1 da.	15.30:9.23 = 1.66
3	2 vs. 1	1 da.	4.50:1.90 = 2.37
3	4 vs. 3	1 da.	4.60:0.30 = 15.33
4	3 vs. 1	20 hr.	5.73:0.07 = 81.86
5	1 vs. 4	1 da.	10.20:0.20 = 51.00
6	1 vs. 2	1 da.	2.00:0.10 = 16.14
7	3 vs. 4	5 hr.	2.40:0.00 = ∞
8	2 vs. (3 + 4)	1 da.	2.90:1.50 = 1.93
9	4 vs. 3	16 hr.	4.40:1.20 = 3.67
10	1 vs. 3	22 hr.	0.25:0.00 = ∞
11	1 vs. 3	1 da.	3.30:1.10 = 3.00
11	2 vs. 3	1 da.	5.10:1.10 = 4.64
12	(2 + 5) vs. 3	2 da.	0.80:0.04 = 20.00
13	1 vs. 3	1 da.	2.40:0.05 = 48.00
13	2 vs. 3	1 da.	1.50:0.05 = 30.00
13	4 vs. 3	1 da.	1.80:0.05 = 36.00
14	2 vs. 1	2 da.	9.60:6.10 = 1.57
15	(1 + 3) vs. 2	22 hr.	3.85:1.80 = 2.14
16	7 vs. 3	2 da.	8.90:3.30 = 2.70

In the 22 comparisons made above, involving a total of 155 chicks in the plasma receptors and 124 chicks in the controls, the ratios of mean counts for the receptors of "immune" (or normal as in Series 2, or still in the primary attack, as in Series 12) duck plasma to the mean counts for the controls, either receptors of physiological salt solution or uninjected, ranged from 1.57 to infinity (∞). The over-all evidence for a significant difference between the two treatments is so preponderant that no special statistical treatment is indicated. Tests for significance, however, were made in a number of the series, of which only several selected examples are submitted.

Series 1 (Fig. 1) was planned to test not only for sparing effects of duck plasma, but also for possible results of delaying injection of parasitized duck erythrocytes into chicks of Groups 3 and 4 for an hour.

after the injection into chicks of Groups 1 and 2 was completed, the duck erythrocyte suspension contained in a beaker at room temperature during the interim. Since data of this sort lend themselves especially well to treatment by analysis of variance, Groups 1 and 2 were tested against Groups 3 and 4 on days 1, 2, and 3 for effects of delaying the injection an hour, and Groups 1 and 3 were tested against 2 and 4 on the same days for the effects of plasma treatment. The F-values obtained indicated that delaying the injections was without effect on days 1, 2,

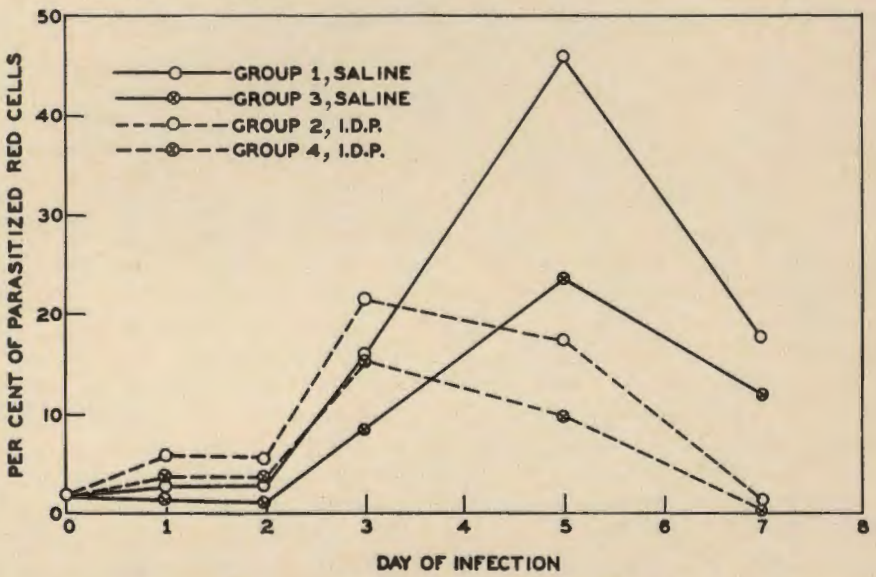

FIG. 1.—Graphic presentation of mean counts in Series 1, comparing saline-receptors of Group 1 with immune duck plasma-receptors of Group 2, and saline-receptors of Group 3 with immune duck plasma-receptors of Group 4.

and 3. An F-value of 3.48 obtained on Day 1 for treatments, however, indicated probability of about 0.08 to be compared with the value 0.05 usually accepted as significant. The tests on Days 5 and 7 indicated that the differences between treatments and between groups, i.e., Gs (1 + 2) vs. Gs (3 + 4), were significant. The conclusions to be drawn are as follows: (1.) Delaying the injections for an hour had little effect during the first three days of the infection, but by the fifth day effects became apparent; (2.) the sparing effect of plasma treatment is suggested but not definitely proved; (3.) the protective effect of the "immune" plasma on Days 5 and 7 was definitely proved.

Series 2, which contained chicks of two ages, was also treated by analysis of variance after being set up in such a way that the 10-hour, 1-day, and 2-day data could be tested collectively for effects of plasma and of age of chick hosts. The values obtained for age were not significant, but those for effect of plasma injection were highly significant. In

this case normal plasma of a grown duck was used instead of immune plasma.

Series 3 was also treated by analysis of variance. Plasma was found to produce a significant sparing effect, but the difference between ages of chicks was not quite significant.

NON-SPECIFIC PROTEIN EFFECTS

If it should be shown that the sparing effect as demonstrated can be induced with a wide variety of protein-containing materials other than duck plasma, then grave doubts would arise as to both its specific nature and immunological significance. Three classes of such materials were tested: (1.) the albumens of fresh chicken, duck, and guinea-fowl eggs; (2.) mammalian blood plasmas from man, rabbit, rat, and guinea pig; (3.) avian plasmas from the chicken, turkey, guinea fowl, and goose.

1. *Egg albumens.* The egg albumens used were first separated from the yolks, mixed with the proper amount of physiological salt solution to make the dilution desired, and beaten a short time with an electric mixer. The mixture was poured into a graduate, and the fluid that collected underneath the foam was used for intravenous injections. Series 8 consisted of 30 chicks, of which 10 were injected with 0.8 cc. of hen's albumen diluted 1:3 with physiological salt solution (about 4.0 per cent protein) on days 0 and 1; 10 injected with the same amount of immune duck plasma; 5 injected with the same amount of physiological salt solution; and 5 were uninjected. The latter ten chicks constituted the control or reference groups. The record shows that the counts for albumen recipients (G 1) and controls (Gs 3 and 4) from the third minute to the thirteenth day of the infection were comparable; in fact, the counts for G 1 on 5 hours, 1 day, and 2 days were somewhat lower than those for Gs 3 and 4. The counts for G 2, recipients of immune duck plasma, however, were much higher during the same periods, especially that at the 2-day interval, which was 11.6 per cent, to be compared with 4.1 per cent for G 1 and 5.4 per cent for Gs 3 and 4. Obviously the albumen possessed no sparing property.

Duck egg albumen was tested in Series 10, G 5 of which received injections of 0.5 cc. of a mixture of 5 cc. albumen and 4 cc. physiological salt solution on Day 0 and 0.7 on Day 2. The controls, G 3, received physiological salt solution. Although it appeared at 3 minutes and 1 hour that a certain degree of sparing action was being produced by the duck albumen, the effect was considerably less evident at 5 hours and 22 hours. The 4-day counts for the two groups were both 0.1. Thus it must be granted that a transient sparing action was in operation, but in the long run the test group came down to the level of the controls. The results in Gs 1 and 2, receptors of immune duck plasma and serum, respectively, were quite different, for here the sparing effects were definitely in evidence from 3 minutes to 7 days after inoculation.

Guinea egg albumen was tested in Series 14 (G 3), 18 (G 1), and 21 (G 2). In these tests also the albumen failed to exhibit significant sparing action, as an inspection of the counts for the albumen-receptors and saline-receptor controls in these groups reveals. The differences in the means of Series 21 appear impressive until the magnitude of the standard deviations is noted.

2. *Mammalian plasmas.* The plasmas were separated from freshly drawn heparinized blood and frozen in the refrigerator a day or two before use. Injections were made intravenously.

Rat plasma was tested in Series 6 (G 3). When the record of G 3, recipients of rat plasma, is compared with that of G 2, controls receiving heparinized physiological salt solution, no conclusion can be drawn other than that the rat plasma produced no sparing effect. When, however, the data for the receptors of immune duck plasma in G 1 are compared with those of Gs 2 and 3 it becomes obvious that the sparing action of the duck plasma was manifesting itself at 1 hour, 5 hours, 1 day, 3 days, 4 days, and 5 days.

Human plasma was tested in Series 10. G 4, the receptors, originally consisted of eight chicks, but two succumbed to the toxic effects of the plasma. The 3-minute, 1-hour, 5-hour, 22-hour, and 4-day counts of G 4 and of the controls, G 3, are so close that it may be assumed no sparing action was exhibited by the human plasma. Gs 1 and 2, however, receptors of immune duck plasma and serum, respectively, did exhibit the sparing effect of the injections.

Guinea pig plasma was tested in Series 15 (G 4) and 18 (G 2). If a comparison is made of the counts over the first two or three days in Gs 4 and 2 of Series 15 and Gs 2 and 3 of Series 18, it becomes obvious that in these cases also no sparing effect was exhibited by the injected plasma.

Rabbit plasma was also tested, though the record does not appear in Table 1. Five chicks were injected with 0.8 cc. of plasma from a white rabbit and five others with physiological salt solution, both an hour and one-half before the injection of 3×10^8 infected duck erythrocytes. The mean counts up to two days for the plasma receptors and saline receptors, respectively, were as follows: 2.5 hours, 0.56 and 0.78; 6 hours, 0.34 and 0.57; 22 hours, 0.80 and 1.04; 2 days, 2.54 and 2.19. Again a mammalian plasma failed to manifest sparing action.

Thus the record of the counts for the receptors of rat, human, guinea pig, and rabbit plasmas, and their controls is very clear in indicating the absence of sparing properties toward infected duck erythrocytes.

3. *Avian plasmas.* The plasmas tested were from chicken, turkey, guinea-fowl, and goose. The bloods were drawn from the right jugular vein, heparinized in sterile screw-cap test tubes, and subsequently treated as described for duck plasma in the material and methods section. All chick injections were intravenous.

Chicken plasmas were tested in Series 5, 9, and 19. Series 19 consisted of four groups of chicks of which G 1 received 0.6 cc. of plasma

pooled from four six-month-old chickens which had been inoculated with *P. lophurae* in chicken blood when 12 days old and given heavy "booster" inoculations when about 4½ months of age. These chickens did not have microscopically demonstrable parasites in their blood when it was drawn. G 2 received normal plasma from a cock of the same hatching as G 1, and G 3 received normal plasma pooled from three 29-day-old male chicks. G 4, the control, received only physiological salt solution. At 2 minutes the mean counts were quite comparable, but at 3 hours, 1 day, 3 days, and 5 days the counts for Gs 1, 2, and 3 were considerably lower than for G 4. The three chicken plasmas obviously possessed no sparing properties, neither the "immune" nor the normal. It appears that it can also be soundly concluded that they carried protective antibodies, especially that from the six-month-old normal cock with which the birds of G 2 were injected.

Gs 1 and 2 of Series 9 both received plasma from chickens which had been infected with *P. lophurae* in chick blood when 60 days of age and, when bled three weeks later, were not carrying microscopically demonstrable infections. From these recovered chicks two groups were sorted out: (1.) four chicks whose record was one of light infections of short duration; (2.) three chicks with a record of comparatively heavy infections with demonstrable parasites persisting in the blood on the eighteenth day, but not on the twenty-first. G 1 received 0.5 cc. of plasma from the first group and G 2 received the same amount from the second, two hours before the injections with the infected duck erythrocytes. At 5 hours, 16 hours, and 2 days the counts for Gs 1 and 2 were significantly less than for G 3, the controls. It is of further interest that at 2 days, 4 days, and 6 days the counts for G 1 and G 2 were significantly different. At first it seemed inconsistent that the plasma from the more resistant recovered chicks conferred a lesser degree of protection than the plasma of less resistant recovered chicks, but upon further consideration the explanation that the antibody concentration in the blood of the chicks of G 1 might have subsided considerably since the infections became latent, while it was probably at or near its peak in the chicks whose infections were the more prolonged, seemed plausible enough. At any rate, neither of the pooled plasmas produced the sparing effect.

Series 5 was of special interest because it was the only one in which sparing action of chick plasma toward duck erythrocytes was observed. The plasma administered to G 2 was separated from the blood of two chicks exsanguinated on the fifty-fifth day of their lives and pooled. These birds had been inoculated with *P. lophurae* in chick cells when twenty-one days of age, and had experienced high parasitemias with 62 and 75 per cent parasitized red cells, respectively, on the fifth day. The parasitemia in one of the chicks was 0.03 per cent on the twenty-second day and in the other 0.5 per cent on the fourteenth day. Thus the donor chicks had entered parasite latency some time after twelve and twenty days, respectively, before the blood was drawn.

These details are given because they may be found later to have some significance in explaining the unique finding that follows.

G 2 (of Series 5) received 0.9 cc. of the pooled chicken plasma two hours before the infected duck erythrocytes were injected, while G 4 received 0.9 cc. of physiological salt solution. At 3 minutes the mean counts for G 2 and G 4 were 3.2 and 3.1, respectively, but at 4.5 hours, 1 day, 3 days, and 5 days, the means for G 2 were obviously significantly higher, though they were higher yet in G 1 whose birds had received immune duck plasma. The mixture of immune chick and immune duck plasmas administered to G 3 resulted in mean counts that were in general intermediate between those for G 1 and G 2. It is obvious that in Series 5 immune chicken plasma injected into chicks exhibited a sparing effect on the removal of duck erythrocytes subsequently injected, though this effect was not so pronounced as that exhibited by the immune duck plasma tested in the same series. It should be recalled at this time that in the previous report (1) there were three series (1, 3, 4) which contained groups of chick injected with immune chick plasma, and that in none of these cases were there indications of sparing effect from the injections.

The normal plasma of the guinea-fowl was tested in Series 16 and 20, and the immune plasma in Series 20. The normal plasma used in Series 16 was from a single hen bird, while that used in Series 20 was pooled from the same bird and one other. The immune plasma used in Series 20 was also pooled from two birds, of which one was in the sixtieth day of its infection and the other in the fifty-fourth. The infections in the latter birds, each of which weighed about 575 grams when inoculated, had been very light, the readings on any one day not exceeding 1.8 per cent parasitized cells in one of the fowls and 0.03 per cent in the other. There were no microscopically demonstrable parasites in the stained blood smears of either bird the morning the blood was drawn.

In Series 16 there were ten chicks (Gs 1 and 4) which received 0.3 cc. of normal guinea plasma two hours before the infected duck erythrocytes were injected, ten chicks which received 0.6 cc. (Gs 2 and 5), two (G 6) which received 1.6 cc. and six (G 3) which received 0.6 cc. of physiological salt solution. There were included, for comparison, three chicks (G 7) receiving 1.6 cc. of immune duck plasma. At 45 minutes the mean count of the controls (G 3) was 1.3, while that of G (1 + 4) was 1.65 and that of G (2 + 5) was 1.95. The differences, while not statistically significant, do indicate a trend, for at 1 day the means for the same groups were respectively, 2.9, 3.95, and 6.1. On the second and fourth days the inequalities between the controls and the two sets of plasma-receptors became even more pronounced. The tendency of the plasma-receptors to become latent earlier than the controls is also indicated by the values appearing in the columns headed by 6 days—16 days. It is obvious that the chicks which received 0.3 and 0.6

cc. of normal guinea plasma exhibited the sparing effect, the latter probably to a slightly greater extent than the former.

Series 20 was planned for retesting normal guinea-fowl plasma and comparing its effects with that of immune guinea-fowl plasma. Groups 2 and 4 received 0.8 cc. of the normal plasma 1½ hours before the infected duck red cells were injected, and G 4 received in addition the same amount of plasma on each of the three following days. Groups 1 and 5 received 0.8 cc. of the immune guinea plasma 1½ hours before the infected duck red cells were injected, and G 5 received in addition the same amount of plasma on each of the following three days. Group 3 received 0.8 cc. of physiological salt solution instead of plasma 1½ hours before the infected duck erythrocytes were injected. At 3 minutes the mean for the eight saline-receptors (G 3) was 1.78, that for the eight receptors of immune plasma (Gs 1 + 5) was 2.78, and that of the eight receptors of normal plasma (Gs 2 + 4) was 2.50. The Fisher small-sample test of the data showed that at 3 minutes the mean of the controls was significantly lower than that of either set of plasma receptors. Since the number of parasitized duck erythrocytes injected into each chick was graded according to the weight of the chick, it is to be concluded that in this case the sparing action of the plasmas was already operating. The disparity was much greater at 5 hours, 1 day, 2 days, and 4 days, and the differences are highly significant.

At 4 days a difference commenced to appear between the mean counts for Gs (1 + 5) and Gs (2 + 4). The P-value obtained was just under 0.05 at this time, but by 5 days the differences between the mean counts for the two sets of chicks had become highly significant (i.e., $P = < 0.01$). This trend held true also on Day 6 and Day 8. Thus the plasma from the immune guinea-fowls asserted protective influence beyond that asserted by normal plasma. The normal plasma seemed to be demonstrating a slight protective effect on Days 6, 8, and 10, as can be judged by comparing Gs (2 + 4) with G 3, the controls.

Goose plasma was tested in Series 22. The donor was a male goose hatched in the spring of 1949 and bled the following December. Ten chicks (G 1) were injected with 0.8 cc. of goose plasma 1½ hours before inoculation with parasitized duck erythrocytes, ten chicks (G 2) received the same treatment except that only half as much of the plasma was injected, and ten controls (G 3) received only the parasitized duck erythrocyes. The mean count of 2.90 per cent parasitized cells made on G 1 three minutes after inoculation indicates the probable minimum initial concentration in the blood of all the groups. After an hour the mean concentrations in G 1 and G 2 were still 2.53 and 2.49, while that in G 3 receded to 1.98. The differences are far from statistically significant, but they do actually indicate a trend, for after 5½ hours the mean concentrations for G 1 and G 2 had risen to 3.15 and 3.65, respectively, owing to invasion of chick erythrocytes by merozoites from duck cells, while in G 3 it was but 1.84. The differences between

the means of G 1 and G 3, and G 2 and G 3 were statistically significant with $P = 0.02$ and $P = < 0.01$, respectively. At the end of one day and two days the differences between G 1 and G 3 were no longer statistically significant, though the mean for G 1 continued higher through the fifth day. The differences between G 2, the recipients of but 0.4 cc. goose plasma, and G 3 were significant at one day and highly significant at 2 days and 3 days, but on the fourth day $P = 0.085$, not considered significant. Thus both groups of plasma-receptors exhibited the sparing effect, the receptors of 0.4 cc. the more markedly.

Although the concentration of parasitized cells in G 1 and G 2 was about the same at 1 hour, afterwards it increased more rapidly in G 2, receptors of but 0.4 cc. goose plasma, than it did in G 1, receptors of 0.8 cc. of the same plasma. At 5½ hours and at 1 day the differences between these groups had not yet become statistically significant, but they were significant at 2 days and at 3 days. At 4, 5, 6, and 8 days the means for G 2 continued higher, though not significantly so. Our interpretation of these data is that G 1, which received twice the amount of goose plasma received by G 2, proceeded to exhibit passively acquired protection from natural, or innate, antibodies after the sparing property of the plasma had manifested itself, while G 2 did not receive enough plasma to show the protective effect so markedly. The explanation for the concentrations being higher in G 1 through Day 5 than in G 3, which received no plasma and hence no passively acquired protection, is undoubtedly the momentum of the higher counts at 5½ hours. Thus the goose demonstrated that is possessed "natural" and hence "nonspecific" antibodies to *P. lophurae*, as well as the sparing property.

Normal turkey plasma from a six-month-old male was tested in Series 17, normal turkey serum from two males of about the same age in Series 23. A comparison of the mean counts of the controls in Series 17 on selected days from 1 to 10 with the related mean counts of the receptors of normal turkey plasma fails to show any significant difference between the counts for the two groups on any day. Series 23, however, yielded altogether different results. In this series eleven chicks (G 1) were injected with 0.4 cc. of turkey serum, ten (G 2) with 0.8 cc. of the same serum, both about 1¾ hours before inoculation with the infected duck erythrocytes. Ten control chicks (G 3) received only the infected duck erythrocytes. The readings of the 45-minute smears showed that removal of the parasitized duck erythrocytes from the circulation had already proceeded much more rapidly in the controls than in the serum-receptors, and the differences were significant. The same was true also on several following days. Thus there is no doubt that in this series the normal turkey serum manifested sparing action.

The question arises whether the larger administration of serum (0.8 cc.) to G 2 produced a heavier infection than the smaller amount (0.4 cc.) administered to G 1. The counts for these groups at 2, 3, and 4 days were so close that the answer is in the negative. It appears that if there were any significant difference between the two groups it was on

Day 6, but the counts within the groups were so variable that the great difference between the means proves not to be statistically significant.

BLOCKING OF THE RETICULO-ENDOTHELIAL SYSTEM

It was pointed out in the previous paper by Becker *et al.* (1) that the sparing effect could not be ascribed to blocking of the reticulo-endothelial system in the ordinary sense because the first injection of immune duck plasma usually produced the effect while the injections repeated on successive days conferred a degree of passively acquired immunity on the host, quite the contrary of what would be expected if the duck plasma were acting as a blocking agent. Another argument that can now be brought against the plausibility of blocking effect is that the mammalian plasmas (man, rabbit, rat, and guinea pig) and egg albumens (hen, guinea, and duck) do not manifest sparing action against infected duck cells in the chick, while the more closely related avian plasmas (duck, guinea, goose, and turkey) do. Still another argument against blocking as an explanation of the sparing effect is the amount of plasma, injected only once, that is sometimes effective. In Series 6, G 1, Series 9, G 4, Series 12, G 1, and Series 14, G 2, single injections of 0.5 cc. of duck plasma produced the sparing effect. In Series 18, G 4, 0.3 cc. of immune duck plasma was not effective, but in a still unpublished experiment significant sparing effect was obtained with a single injection of 0.15 cc. of immune plasma per 100 g. of chick body weight.

Attention should also be directed to Series 20 wherein Gs 1 and 2 received but a single injection of 0.8 cc. of guinea plasma two hours before injection of the parasitized duck red cells and Gs 4 and 5 received the same amount at the same time and again on Days 1, 2, and 3. An inspection of the means and standard deviations throughout the tests shows that the birds receiving the three additional injections were no more susceptible than those receiving the single injection, an observation not in conformity with the well-known cumulative effects of repeated injections of foreign materials in producing blocking.

TOXICITY OF DUCK PLASMA

If the sparing effect were attributable to a general breakdown of the host's resistance produced by the toxicity of duck plasma, other manifestations such as inappetence and consequent failure to grow or loss of weight should appear. In order to control this possibility, all of the chicks used as hosts in these experiments were weighed just before the injections commenced and again on the next, second or third days, and at later times during the course of the infection. These weights have been studied for indications of weight disturbances in the receptors of duck plasma that manifested the sparing reaction, with the result that no evidence was found which suggested that the plasma-receptors in general made less growth gains than the controls which either received saline solutions or were uninjected. This statement applies only during

the first two or three days of the infection, however, for the weights of the plasma-receptors are often affected on the fourth to seventh days while the growth of the controls may be still unaffected. The explanation for these weight losses lies in the heavy infections that develop earlier in the plasma-receptors on account of the momentum of the larger number of parasitized duck cells that escape removal from the circulation by the clearance mechanism.

Another argument against a hypothetical toxicity explanation of sparing action is that the further removed (i.e., from chicks) plasmas of mammals, which might be expected to produce the effect in an even more accentuated form than bird plasmas, manifested no sparing properties at all.

THE LAG PHASE IN REPRODUCTION OF THE PARASITE

It has been suggested that the observed differences between the counts for the plasma-receptors and controls might be attributable to duck plasma reducing the lag phase of reproduction of the parasites passed from the duck into the chick, rather than to a role in preventing the removal of the infected duck erythrocytes from the circulation. While we do not hold against the possibility that duck plasma may in some way support the growth and reproduction of the parasites from duck blood during their early development in the chick, there is in the table the most convincing evidence that the observed phenomenon is a genuine sparing effect. In Series 4, for example, each of the chicks constituting Gs 1, 2, and 3 was injected intravenously with the same volume of suspended duck cells per 100 g. of body weight from the same dilution which, according to our practice, was thoroughly mixed by pouring from beaker to beaker between individual injections. At 3 minutes after inoculation the average of the mean counts for the 10 chicks in G 1 and the 10 chicks in G 2, receptors of physiological salt solution and heparinized physiological salt solution, respectively, was but half the mean count for the 10 chicks in G 3, so fast had removal of the parasitized duck cells from the chick's circulation already proceeded. The differences are statistically significant. At 4.5 hours the removal of the parasitized cells from the chicks' blood had proceeded so much more rapidly in Gs 1 and 2 than in G 3 that the ratio became 1:50.

While it is true that removal of the infected duck cells proceeded at an unusually rapid rate in the controls of this series and of Series 10 also, a similar though not so rapid removal occurred in the controls of Series 3, 5, 6, 7 , 9, 11, 12, and 13. In all of these cases the mean counts of the controls underwent during the first day or two a tremendous reduction from the probable initial count after injection. It is of further significance that the receptors of immune plasma did not uniformly experience an important increase in parasite numbers during the first 24 hours, for some of them either about held their own or experienced modest to rather considerable declines. Thus the record is plain in

indicating that duck plasma spared the infected duck erythrocytes from rapid destruction throughout the first day or so after their injection into the chick, and does not lend itself to interpretation in favor of its role in reducing a lag period in the parasite's multiplication. As was intimated above, however, the authors are not going on record as stating that the plasma cannot play the latter role, but even if it can such activity should not be confused with the sparing phenomenon.

HEMOLYSIS

If duck plasma injected into chicks in the amounts employed should produce extensive hemolysis of the chick cells during the first day or so, then the per cent of parasitized duck cells introduced into the circulation would constitute an inordinate per cent of the total red cells of the chicks' blood, and the sparing effect would be purely illusory. It is evident that such was not the case, for the appearance of inordinate numbers of basophil erythroblasts and polychromatophil erythroblasts that follows the hemolytic process in birds was not observed during the first hours or day or two of the manifestation of the sparing effect.

In order to check more accurately on the possibility of hemolysis, total red blood cell counts were made at selected times. The counts for Series 4, in which the sparing effect was pronounced, may be submitted as typical. The mean counts two days before the injections were as follows: G 1, 258 x 10^4; G 3, 251 x 10^4. On Day 3, by which time the sparing effect was still strongly indicated, they were as follows: G 1, 263 x 10^4; G 3, 225 x 10^4. Thus there was little difference between the red blood cell counts either before the injections or on Day 3; and the slight differences between the red blood cell counts on Day 3 could not account for the great differences between the parasite counts. On Day 5, by which time enormous numbers of parasitized cells had been cleared, the counts were as follows: G 1, 251 x 10^4; G 3, 153 x 10^4. Thus the heavier parasitism in G 3 was taking a heavy toll of the chicks' red cells. By Day 10, however, the situation had almost become reversed, and the counts were as follows: G 1, 150 x 10^4; G 3, 191 x 10^4. The change was due to the fact that the reproduction of the parasite in G 1 finally caught up, while the parasitemias in G 3 had declined and most of the destroyed erythrocytes had been restored.

THE ROLE OF THE ANTICOAGULANT

There was one experiment in the previous paper (1) which showed that sodium citrate used as the anticoagulant did not produce the sparing effect. The anticoagulant most generally used, however, was heparin, and it was the only anticoagulant used in preparing the plasmas for the injections made in the present work. Tests for its effect were made in two ways: (1.) injecting a solution in physiological salt solution of the same concentration of heparin as the heparinized plasma (Series 4 and 6); and (2.) injecting serum from the clot in place of plasma (Series 10 and 14). In Series 4, G 1 received physiological salt solution,

G 2 a solution of heparin in physiological salt solution and G 3 immune duck plasma for the preparation of which heparin was used as the anticoagulant. It is obvious that the quantitative characters of the infections in Gs 1 and 2 were comparable throughout, considering the standard deviations. G 3, however, showed a highly significant sparing effect.

In Series 6, G 1 received immune duck plasma and G 2 the solution of heparin in physiological salt solution. At 3 minutes the two groups were comparable, but at intervals of from 1 hour to 5 days the means for G 1 were significantly higher. In Series 10, G 1, composed of recipients of heparinized plasma, is to be compared with G 2, recipients of serum from the clot. Inspection of the means and their standard deviations reveals no difference between the two groups. If the data for these groups are compared with those of G 3, recipients of physiological salt solution, it becomes evident that the plasma and serum both produced the sparing effect. In Series 14, G 2, immune duck serum was employed for the test instead of the plasma. In this case also the sparing effect was evident. Thus heparin as an anticoagulant is cleared of responsibility for the sparing effect.

DISCUSSION

An imposing array of data has been mustered to support the validity of the sparing effect of duck plasma and serum, whether immune, normal, or from birds in the primary attack. One of the most impressive features of these new data is the difference between test and control groups at the time of the first count. The means of the first counts, based on smears made at one minute to 50 minutes after injection of the duck erythrocytes, were at least some degree higher in plasma- or serum-receptors than in the controls in every one of the following series: 1-minute smears, Series 9; 2-minute, Series 18; 3-minute, Series 3, 4, 6, 7, 10; 5-minute, Series 1, 2; 20-minute, Series 13, 14; 45- to 50-minute, Series 15, 16. In Series 11 the mean for G 2, plasma-receptors, was but slightly higher than that of the controls (G 3) at 2 minutes, but that of G 1, also plasma-receptors, was slightly lower than that of G 3, though it was much higher at one hour. In Series 8, G 2 and G 3 had identical mean counts at 3 minutes, but at one hour the mean of G 3 (controls) had declined the more. In Series 5, duck plasma-receptors, G 1 and G 3 had means of 3.1 and 3.7, respectively, while the controls (G 4) had a means of 3.1, but after 4.5 hours the controls were far below the others. Thus there were already definite indications of the sparing effect in every series at one to 50 minutes after injection of the infected duck erythrocytes, save for the three groups of three series which did, however, show the sparing effect one to 4½ hours later.

The sparing effect is, of course, the result of plasma (or serum) action in reducing the destruction of the parasitized cells. It is the

washed parasitized duck cells, playing the role of foreign bodies in the chick's circulation, which are destroyed during the first period of about 36 hours after inoculation. (According to Hewitt (5), each asexual generation of *P. lophurae* requires about 36 hours.) These are removed from the blood of individual chicks at extremely variable rates, a statement which is supported by a study of the ratio of the standard deviations to the means appearing in the table. It is of further interest that the disappearance rate is in general more variable in the controls than in the plasma-receptors. A considerable number of the parasitized duck cells are destroyed in most of the control chicks, all but a microscopically undemonstrable residue in a few of them, but practically all escape destruction in still others. It is these facts that make the standard deviations in the controls so variable. On the other hand, the sparing effect is not so marked in some plasma-receptors as it is in others, a situation which makes for high standard deviations in groups where such individual differences are frequent.

It must be assumed that the major factor in removal of the parasitized duck erythrocytes from the blood of the chicks was phagocytosis by macrophages in the spleen, liver, bone marrow and other organs. The spleens of birds are comparatively small and the livers comparatively large, but both organs function importantly in normal erythrophagocytosis (Maximow and Bloom (6), pp. 98–99), and would be expected to do so in phagocytosis under the conditions of these experiments also. To what extent haemolysis was concerned remains unknown.

The livers and spleen of two control chicks in which rapid disappearance of the duck cells was occurring were removed after one hour, fixed in Bouin's fluid, sectioned at 5 microns, and stained in Delafield's hematoxylin. Considerable numbers of v. Kupffer cells were observed in the liver sections with ingested and adhering erythrocytes containing malarial pigment. The splenic cells did not show much comparable activity, though our study was not complete.

It is only fair that inquiry be made concerning the fate of the nonparasitized duck erythrocytes. It has not been possible to distinguish between the normal chick and duck erythrocytes in the stained blood smears. Cell dimensions and smoothness of the nuclear membranes were set up as tentative morphological criteria for making the distinction, but both were too fallible for practical use. Other methods will have to be sought for such a study. Nucleated erythrocytes not containing malarial parasites were observed in v. Kupffer cells in the liver sections referred to above, but it was not possible to distinguish between those of duck and chick origin.

Another important consideration is that the sparing phenomenon is related to innate or natural immunity rather than to acquired immunity, for it is the operation of the chick's basic defense mechanism against the duck cells labelled with malarial parasites that is inhibited. It appears that duck plasma may contain an inhibiting antibody or principle

that in some way or other neutralizes natural opsonins in the chick's blood. This point is being emphasized because Taliaferro and Taliaferro (9) have stressed that treatment with nitrogen mustard, X-raying, and blockade with foreign red cells (Gingrich) produce injury to the acquired parasiticidal mechanism superimposed on innate immunity, but never break through and affect innate immunity.

In actively acquired immunity to bird malaria (i.e., parasitic latency) the host, if injected intravenously with washed red cells infected with the homologous strain of parasite and of the same bird species, rapidly clears its blood of the parasitized cells, whereas in a normal bird infection results (Taliaferro and Taliaferro (10). The removal of the parasitized erythrocytes from the latent bird "is due mainly to an actual ingestion and digestion of the parasite-erythrocyte combinations by the phagocytic cells of the spleen and liver" (Cannon and Taliaferro (2). The reason advanced for the increased rate of phagocytosis in an immune (latent) bird is both the possession of a greater number of phagocytic cells and functional acceleration of the individual phagocytes.

A certain degree of phagocytosis of parasitized erythrocytes occurs in a bird host during the primary attack, however, as has been proved by the work of L. G. Taliaferro (8) and Hartman (3) in connection with that of Cannon and Taliaferro (loc. cit.). Taliaferro found by means of an ingenious formula that in the primary attack of *Plasmodium cathemerium* ("praecox") infection in canaries 5.05 merozoites survived to complete their development during each generation of 24 hours length, whereas each segmenter had actually liberated 15.5 merozoites. The parasite mortality in the normal bird was then about 67 per cent. Hartman (3), attacking the same problem in a different but equally ingenous way, determined by fit of his data to an exponential curve that the same parasite (i.e., *P. cathemerium,* also in canaries) had a constant death rate during at least 21 hours of the 24-hour asexual generation. The mortality per hour was 5.184 per cent, 3.875 per cent, and 3.507 per cent in three birds studied. Cannon and Taliaferro observed that parasites (inside red cells) which disappeared from the circulation under these conditions are phagocytosed, principally in the spleen and liver, but these authors did not commit themselves concerning the viability of the parasites at the same time they were engulfed. Similar findings, not reviewed here, have been reported by the Taliaferros and their collaborators in infections of other *Plasmodium* species in other hosts.

It is true that phagocytosis as it occurred in the control chicks was not the product of actively acquired immunity, and on the surface at least was too rapid to be considered qualitatively identical with that responsible for the constant death-rates noted during the primary attack in several species of malarial infection. Nevertheless, it has this in common with the others, in phagocytosis during both actively acquired

and innate immunity the phagocytosing cell recognizes the homologous parasitized erythrocyte as a foreign body, and that is what occurs also when parasitized heterologous cells are injected into young chicks.

It should be stated, parenthetically, that in still unpublished investigations we have shown that phagocytosis proceeds more rapidly in chicks of four weeks of age or older, and that in such older chicks it is impossible to arrest consistently the removal of the duck cells with duck plasma, at least in the amounts effective in the younger chicks employed in this investigation.

The investigation has brought out that the sparing phenomenon cannot be interpreted as (1.) a non-specific protein effect, (2.) an illusion due to hemolysis, (3.) blocking of the macrophages in the ordinary sense, (4.) a state of intoxication of the host produced by heterologous plasma, (5.) an effect produced by the anticoagulant, or (6.) a reduction of the lag phase in reproduction of a parasite not yet adapted to its new specific host, brought about by an unknown but essential (to the parasite) quality of duck plasma. Since the facts do not bear a possible alternative explanation, it must be regarded as specific. Not species specific, for plasma of the normal and immune guinea-fowl, normal goose, normal turkey and, in one test, normal chicken also produced the sparing effect, while mammalian plasmas did not. The sparing property of these bird plasmas was just as specific as the property of immune duck and guinea-fowl plasma that conferred partial protection on the chick after the parasites had transferred to the chick's own red cells.

The occurrence of antibodies in the plasma of the normal duck, goose, and chicken brings up the matter of so-called natural antibodies. Such plasmas, when injected into chicks, can confer a partial passively acquired immunity on the chick of a character not unlike that conferred by duck plasma from recovered ducks. If the general concepts of Pauling (7) and Haurowitz (4) concerning the nature of antibodies are sound, categorical distinction between natural and actively acquired immunity has lost its fundamental significance, for antibodies differ from normal serum globulins only in the physical configuration of certain parts of the polypeptide chain. In a molecule of actively acquired antibody this configuration would be the complement of certain surface areas of the antigen. It is reasonable to assume that in a molecule of natural antibody the same configuration may have arisen through the complementing of the surface areas (templates) of other substances fortuitously like those of the antigen.

Trager and McGhee (11) recently reported that plasma of normal adult chickens contains a material that, when injected into young chicks infected with *Plasmodium lophurae,* is capable of depressing the multiplication of the parasites. Still more recently the same authors have found that about one-fourth of the adult ducks tested produced plasma which reduced the parasite's multiplication in ducklings. We also have made similar studies with immune and normal duck plasma

in ducklings, previously mentioned (1), that we hope to publish in the near future, and it cannot be said that they contradict those of Trager and McGhee.

CONCLUSIONS

1. Additional experiments with slightly improved procedures have provided further and incontrovertible proof of the sparing effect of normal and immune duck plasmas on duck erythrocytes parasitized with *Plasmodium lophurae* against destruction when injected into the blood stream of young chicks.

2. The sparing effect is not produced by blocking of the reticulo-endothelial system with the injected plasma.

3. The sparing effect is not merely the result of the presence of foreign protein per se in the blood of the chick.

4. The sparing effect is not the result of a state of intoxication produced by a foreign protein.

5. The explanation of the sparing effect is not hemolysis of the chick's cells by the duck plasma and survival of the parasitized duck cells.

6. The sparing effect is not reduction of a lag phase in the reproduction of the parasite following introduction of the parasite to a new host.

7. The sparing effect is not due to the presence of heparin, the anticoagulant, in the duck plasma.

8. Normal and immune guinea-fowl plasma, normal goose plasma, normal turkey serum, and normal chicken plasma (in one test only) spared washed parasitized duck erythrocytes from destruction in the chick's blood stream.

9. Immune guinea-fowl plasma, normal goose plasma and, in some instances, normal chicken plasma conferred partial protection on the chick during the primary attack that had its origin in parasites introduced with the parasitized duck cells.

10. Pooled plasma from normal ducklings aged two days (Series 9), five days, and ten days (Series 7) produced the sparing effect, but not passive immunity, when injected into young chicks. The effect was weaker than when "immune" plasma from older ducks was employed.

11. It is the chick's innate resistance to the introduced parasitized red cells that is affected in the sparing phenomenon.

12. The property of bird plasma producing the sparing effect may be an inhibitory antibody, though other plausible explanations also suggest themselves.

LITERATURE CITED

1. Becker, E. R., C. E. Brodine, A. A. Marousek, and D. Byrd.
1949. Influence of normal and immune duck plasmas on chick infections of *Plasmodium lophurae* induced with parasites in duck erythrocytes. Iowa State Coll. Jour. Sci. 23:323–41.

2. CANNON, P. R. AND W. H. TALIAFERRO
1931. Acquired immunity in avian malaria. III. Cellular reactions in infection and superinfection. Jour. Prev. Med. 5:37–64. 1 pl.

3. HARTMAN, E.
1927. Certain relationships between *Plasmodium praecox* and its host. Am. Jour. Hyg. 7:407–32.

4. HAUROWITZ, F.
1949. Biological problems and immunochemistry. Quar. Rev. Biol. 24:93–101.

5. HEWITT, R.
1942. Studies on the host-parasite relationships of untreated infections with *Plasmodium lophurae* in ducks. Am. Jour. Hyg. 36:6–40.

6. MAXIMOW, A. R. AND W. BLOOM
1948. A textbook of histology. 5th Ed. Philadelphia.

7. PAULING, L.
1940. A theory of the structure and process of formation of antibodies. Jour. Am. Chem. Soc. 62:2643–57.

8. TALIAFERRO, L. G.
1925. Infection and resistance in bird malaria, with special reference to periodicity and rate of reproduction of the parasite. Am. Jour. Hyg. 5:742–89.

9. TALIAFERRO, W. H. AND L. G. TALIAFERRO
1948. Reduction in immunity in chicken malaria following treatment with nitrogen mustard. Jour. Inf. Dis. 82:5–30.

10. ——— AND ———
1929. Acquired immunity in avian malaria. Jour. Prev. Med. 3:197–223.

11. TRAGER, W. AND R. B. MCGHEE
1949. Passive transfer of age resistance to an avian malaria parasite. Federated Proc. 8 (1): 373.

12. ——— AND ———
1949. The antimalarial activity of the plasma of certain adult ducks against *Plasmodium lophurae*. Jour. Parasit. 35 (Supplement):24.

PHANEROZOITES IN TURKEYS SUCCUMBING WITH BLOOD-INDUCED *PLASMODIUM LOPHURAE* INFECTION [1]

ELERY R. BECKER AND MIGUEL MANRESA, JR.

Department of Zoology and Entomology
Iowa State College

Received April 12, 1950

According to Taliaferro and Taliaferro (4), Porter and Laird in a personal communication reported that they had not found exoerythrocytic forms of *Plasmodium lophurae* in blood-induced infections of *Plasmodium lophurae*. Huff (2) did not list *P. lophurae* among species of *Plasmodium* in which phanerozoites were known. The term phanerozoites was proposed by Huff and Coulston (1) for exoerythrocytic stages of malaria parasites which occur late in the infection and not as one of the pre-erythrocytic stages.

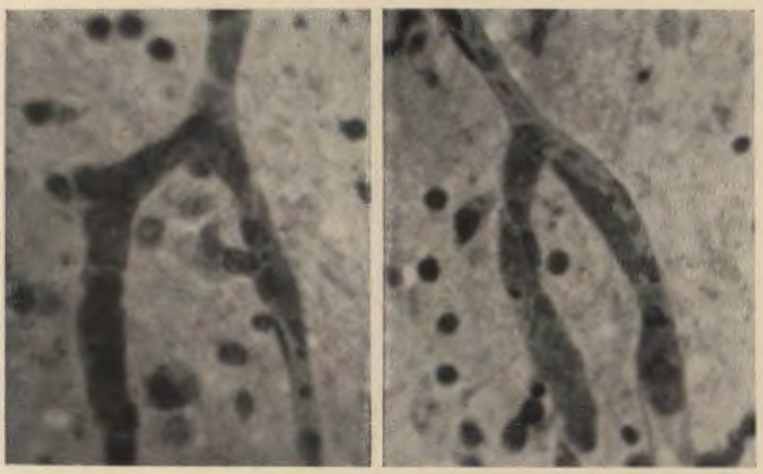

FIG. 1.—Capillaries with phanerozoites of *Plasmodium lophurae*. x500

The present authors have found phanerozoites in brain smears of two 46-day-old turkeys which succumbed on the twenty-first day of infection with *P. lophurae*. The infections were induced with 5 x 10^7 parasitized turkey erythrocytes per 100 g. of body weight. The percentage of parasitized cells attained 56 per cent and 40 per cent, respectively, in the two infections on the sixth day, then subsided, attained new peaks

[1] This investigation was supported in part by Research Grant 605 (C) from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service.

of 50 per cent and 39 per cent, respectively, on the eleventh day, and subsided to 0 per cent on the seventeenth and eighteenth days. A few parasites were seen in the blood again on the nineteenth day. The turkeys were found dead the morning of the twenty-first day. Post-mortem blood smears showed a parasitized cell incidence of 1.83 per cent and 2.70 per cent, respectively.

Smears of the brains of the deceased birds were fixed in methyl alcohol and stained in Giemsa. Phanerozoites measuring from 7.1 μ x 19.4 μ to 13.0 μ x 69.3 μ were observed in the capillaries (Fig. 1). The capillaries were obviously occluded by the larger parasites, and in some cases were definitely distended by restrained erythrocytes. The relationship of the parasite to the capillary was very much like that observed by James and Tate (1938) in *P. gallinaceum* infection of chickens. Both presegmenters and segmenters were observed.

LITERATURE CITED

1. Huff, C. G. and Coulston, F.
1946. The relation of natural and acquired immunity of various avian hosts to the cryptozoites and metacryptozoites of *Plasmodium gallinaceum* and *Plasmodium relictum*. Jour. Inf. Dis. 78: 99–117.

2. Huff, C. G.
1948. Exoerythrocytic stages of malarial parasites. Am. Jour. Trop. Med. 28: 527–31.

3. James, S. P. and Tate, P.
1938. Exo-erythrocytic schizogony in *Plasmodium gallinaceum* Brumpt, 1935. Parasitology. 30: 128–38.

4. Taliaferro, W. H. and Taliaferro, L. G.
1945. Immunological relationships of *Plasmodium gallinaceum* and *Plasmodium lophurae*. Jour. Inf. Dis. 77: 224–48.

THE NORMAL HEMOCYTE PICTURE OF THE YELLOW MEALWORM, *TENEBRIO MOLITOR* LINNAEUS[1]

JACK COLVARD JONES

U. S. Public Health Service, Bethesda, Md.

Received February 17, 1950

Hemocytes (hemolymph cells) of the common yellow mealworm, *Tenebrio molitor* Linnaeus (Coleoptera), may be separated into eight main classes and twenty-three sub-types of variant cells by modification of a classification used by Yeager (5) for hemocytes of the southern armyworm, *Prodenia eridania* (Cram.) (Lepidoptera).

Though *Tenebrio* and *Prodenia* differ widely systematically, the fundamental similarity of certain of their hemocyte classes is apparent. However, variations in the occurrences of these classes, and fluctuations in their numbers during the respective life spans are significantly different. Cell classes and their numbers are considerably more stable in *Tenebrio* than in *Prodenia*.

Ranges of cell and nuclear dimensions of the twenty-three subtypes; and normal differential hemocyte counts of the eight classes throughout the life span of the mealworm are presented. Such counts provide one basis for further studies on the normal and abnormal physiology of this insect. A scheme of developmental relationships among the hemocyte classes is also suggested.

METHODS

A total of 1,364 mealworms was used in this study. Included were 1,202 larvae, from 3 to 33 millimeters in length; 44 pupae, from less than 1 hour to 7 days in age; and 118 adults, from less than 1 hour to 14 days in age. The colony was maintained at 25° to 30°C. on whole wheat bran and sweet potatoes. Before taking hemolymph samples, specimens were submerged in a water bath at 55° to 60°C. for 1 to 2 minutes to heat fix the hemocytes. A total of 142,400 hemocytes was classified from smears of air dried hemolymph stained with Wright's blood stain (1 minute undiluted; 30 minutes diluted). Only class counts were made. From 100 to 1,000 cells were counted per smear. Instars could not be accurately identified; from 1 to 93 individuals were used for a given larval measurement.

RESULTS

Hemocytes of *Tenebrio molitor* were differentiated by combining the following data: (1.) nuclear-cytoplasmic dimensions, (2.) optical

[1] Taken in part from a thesis submitted by the author to the Graduate School of Alabama Polytechnic Institute, Auburn, Alabama, in partial fulfillment of the requirements for the degree of master of science.

appearance of cytoplasm, (3.) type and degree of cytoplasmic inclusions, (4.) cell shape, and (5.) staining reactions.

Eight classes of hemocytes of the mealworm are named as follows: (I) prohemocytoids; (II) smooth-contour-chromophilic cells; (III) oenocyte-like cells; (IV) plasmatocytes; (V) vermiform cells[2]; (VI) cystocytes, or coarsely granular hemocytes; (VII) spheroidocytes; and (VIII) degenerating cells (see Fig. 1). These classes may be further divided into varying numbers of somewhat arbitrary sub-types:

Prohemocytoids: (1.) microcytes and (2.) prohemocytes.

Smooth-contour chromophilic cells: (3.) liocytes and (4.) liocytoids.

Oenocyte-like cells: (5.) pseudoenocytoid and (6.) oenocytoid.

Plasmatocytes: (7.) eoplasmatocytes, (8.) eoplasmatocytoids, (9.) microplasmatocytes, (10.) mesoplasmatocytes, (11.) macroplasmatocytes,

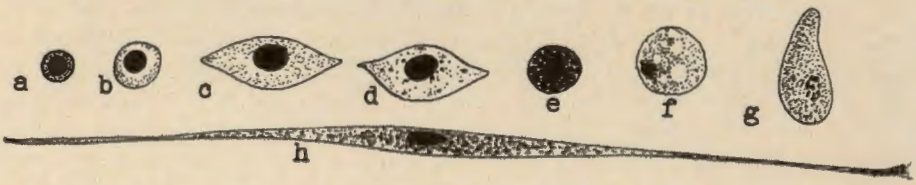

FIG. 1.—Examples of the more important hemocytes of *Tenebrio molitor;* a, prohemocytoid; b, smooth-contour chromophilic cell; c, agranular plasmatocyte; d, granular plasmatocyte; e, cystocyte (coarsely granular hemocyte); f, spheroidocyte; g, oenocyte-like cell; h, vermiform cell.

(12.) elongated plasmatocytes, and (13.) multiramous plasmatocytes.

Vermiform cells: (14.) nematocytoids.

Cystocytes, or coarsely granular hemocytes: (15.) cystocytoids, (16.) neocystocytes, and (17.) paleocystocytes.

Spheroidocytes: (18.) spheroidocytoids, (19.) orthospheroidocytes, and (20.) metaspheroidocytes.

Degenerating cells: (21.) achromophiles, (22.) macronucleocytic degenerating cells, and (23.) disintegrating cells. Cell measurements for the various sub-types are given in Table 1. General descriptions of these cells may be found in Yeager's classification (5).

Most of the class relationships among *Prodenia* hemocytes as schematized by Yeager (5) seem to apply to mealworm hemocytes. Thus the prohemocytoids, which divide by mitosis more frequently than do any other cell class, are considered to be the stem cell for six, and, perhaps, all seven of the other hemocytes classes. Smooth-contour chromophilic cells appear to be transitional cells from which plasmatocytes, cystocytes, spheroidocytes, and, possibly, oenocyte-like cells develop. Plasmatocytes appear to differentiate into cystocytes through progressive decrease of cytoplasmic basophilia, and by a concurrent or

[2] The designation "vermiform cells" is a tentative one. They differ from the elongated plasmatocytes in length and are generally intensely basophilic. It may be that they are no more than extremely elongated plasmatocytes.

subsequent acquisition (maturation ?) of granules into large eosinophilic inclusions.

None of the hemocyte classes of the mealworm was found to divide by amitosis. Neither multinucleate nor binucleate hemocytes were encountered.

The following cell types are very rare in the hemolymph of the normal mealworm: eoplasmatocytes, eoplasmatocytoids, multiramous plasmatocytes, nematocytoids, and achromophiles.

TABLE 1

RANGE OF CELL AND NUCLEAR DIMENSIONS IN MICRONS FOR THE HEMOCYTE TYPES OF *Tenebrio molitor*

Hemoctye Types	Range of Cell Size		Range of Nuclear Size	
	Minimum	Maximum	Minimum	Maximum
1. Microcytes	3.0 x 3.0	7.5 x 3.0	2.0 x 2.0	4.5 x 3.0
2. Prohemocytes	5.2 x 3.0	7.5 x 4.5	3.0 x 3.0	4.5 x 3.0
3. Liocytes	6.0 x 3.5	9.0 x 4.5	3.5 x 3.0	3.5 x 3.5
4. Liocytoids	7.5 x 3.0	10.0 x 4.5	3.0 x 3.0	4.5 x 3.0
5. Pseudoenocytoids	20.0 x 10.0	21.0 x 11.0	4.5 x 4.5	6.0 x 6.0
6. Oenocytoids	7.5 x 7.5	18.0 x 9.5	3.0 x 3.0	7.5 x 3.7
7. Eoplasmatocytes	6.0 x 6.0	10.5 x 9.0	3.0 x 3.0	4.5 x 4.5
8. Eoplasmatocytoids	.....	.....	.....	..... *
9. Microplasmatocytes	6.0 x 6.0	12.0 x 3.0	3.0 x 2.2	4.5 x 3.0
10. Mesoplasmatocytes	9.0 x 7.5	21.0 x 4.5	3.0 x 3.0	6.0 x 4.5
11. Macroplasmatocytes	15.0 x 12.0	25.0 x 18.0	4.5 x 4.5	12.0 x 7.5
12. Elongated plasmatocytes	30.0 x 11.5	42.0 x 5.2	3.7 x 3.0	6.0 x 2.2
13. Multiramous plasmatocytes	17.5 x 17.5	30.0 x 7.5	3.7 x 3.7	6.0 x 4.5
14. Nematocytoids	100.0 x 5.0	132.0 x 4.5	4.5 x 3.0	5.2 x 3.0
15. Cystocytoids	4.5 x 4.5	9.5 x 6.7	3.0 x 3.0	4.5 x 4.5
16. Neocystocytes	6.0 x 6.0	7.5 x 6.0	3.0 x 3.0	4.5 x 3.0
17. Paleocystocytes	7.5 x 3.5	10.5 x 5.2	3.0 x 1.5	4.5 x 3.0
18. Spheroidocytoids	4.5 x 4.5	7.5 x 7.5	3.3 x 3.0	3.7 x 3.0
19. Orthospheroidocytes	9.7 x 4.8	10.8 x 5.4	3.0 x 3.0	4.5 x 3.0
20. Metaspheroidocytes	10.5 x 7.5	13.5 x 11.2	4.5 x 3.7	6.0 x 6.0
21. Achromophiles	.....	9.0 x 7.5	.....	3.0 x 2.2
22. Macronucleocytic degenerating cells	9.7 x 7.5	22.0 x 18.0	6.7 x 6.0	12.0 x 12.0
23. Disintegrating cells	.....	.....	.....	..... *

* No cell measurements taken.

The prohemocytoids (proleucocytoids of *Prodenia*), smooth-contour chromophilic cells, oenocyte-like cells, plasmatocytes, vermiform cells, and degenerating cells are fundamentally similar to *Prodenia* hemocyte classes of the same names. None of the hemolymph cells of the mealworm resemble either the podocytes or the eruptive cells of *Prodenia*. The cystocytes (coarsely granular hemocytes) and spheroidocytes of *Tenebrio* are not exact equivalents of those described for *Prodenia*. Consequently, brief descriptive differences between these are given below.

Yeager (5) suggested that granular hemocytes of other insects might be comparable to *Prodenia* spheroidocytes. The coarsely granular hemocytes (cystocytes) of *Tenebrio* do not, however, fit descriptions for *Prodenia* spheroidocytes. Cystocytes of *Tenebrio* are small to medium in size and contain few to numerous, distinct, round, eosinophilic inclusions in a generally hyaline cytoplasmic envelope. Cystocytoids have few inclusions; neocystocytes have many inclusions, but these are not compact and do not fill up the cytoplasmic envelope; and paleocystocytes have their cytoplasmic envelope completely filled with eosinophilic inclusions.

Spheroidocytes of *Tenebrio* are small to medium in size, contain few to many generally distinct, round, colorless cytoplasmic vacuoles, and have small eccentric nuclei.

As shown in Table 2, plasmatocytes and cystocytes account for more than 90 per cent of the hemocytes encountered in the hemolymph of the mealworm throughout its life span. The other six classes, while they make up only a small fraction of the cells seen are, nevertheless, an essential part of the hemocyte picture.

DISCUSSION

While variations in the differential counts of the eight hemocyte classes in the mealworm are neither striking, nor complexly interrelated as they are in the southern armyworm, minor shifts in their percentages tend to characterize certain stages of development of the mealworm. Larvae, 3 to 15 millimeters in length, show relatively higher percentages of prohemocytoids, plasmatocytes, and degenerating cells than do larvae of other lengths. Larvae that measure 16 to 25 millimeters in length contain higher percentages of smooth-contour chromophilic cells and cystocytes and show relatively lower percentages of prohemocytoids, oenocyte-like cells, spheroidocytes, and degenerating cells than do other larvae. Larger larvae, measuring 26 to 33 millimeters in length, have significantly higher percentages of oenocyte-like cells and spheroidocytes than do larvae of other sizes.

The pupal stage is characterized by lower percentages of prohemocytoids, smooth-contour chromophilic cells, and oenocyte-like cells than those of the larval stage, and by a significantly higher percentage of degenerating cells than found in any other stage of development. Nematocytoids (vermiform cell class) appear only at the very beginning of the pupal stage, but are not invariably present even then. Pupal hemolymph smears generally contain some tissue debris, many plastids, and often free-fat globules of widely varying size. Perez noted in his study of *Calliphora* that fat cells were liberated in the hemolymph in 30-hour old pupae (2).

In mealworm pupae the free flowing peripheral hemolymph stream, although difficult to sample is, when properly taken, much less degenerative in character than that of *Prodenia* pupae. While the number of peripherally circulating hemocytes appears to be markedly lowered

during the pupal stage, histological evidence indicates that the actual total number of hemocytes present within the body is not significantly reduced; most of the hemocytes during this phase are confined to the internal interstices of metamorphosing tissues.

Adult hemolymph contains relatively fewer prohemocytoids and smooth-contour chromophilic cells than does that of other stages. Oenocyte-like cells in the adult mealworm do not disappear, although

TABLE 2

NORMAL DIFFERENTIAL HEMOCYTE CLASS COUNTS OF *Tenebrio molitor*

Stage	No. Insects Used	% Hemocyte Classes*								No. Cells Counted
		I	II	III	IV	V	VI	VII	VIII	
3 mm. larvae...	1	0.6	0.0	0.0	39.4	0	59.0	0.6	0.4	500
4 mm. larvae...	8	0.6	1.8	1.3	38.5	0	52.4	1.8	3.6	800
5 mm. larvae...	23	1.8	2.6	0.5	43.9	0	50.0	0.5	0.7	2,300
6 mm. larvae...	21	2.1	2.4	0.8	43.4	0	51.1	0.1	0.1	2,100
7 mm. larvae...	35	0.9	1.0	0.6	43.5	0	53.3	0.4	0.3	3,800
8 mm. larvae...	68	1.5	2.0	0.4	44.8	0	50.6	0.5	0.2	6,800
9 mm. larvae...	57	0.9	2.1	0.7	48.0	0	48.0	0.2	0.1	5,700
10 mm. larvae...	48	0.5	0.9	1.1	41.4	0	53.2	1.6	1.3	4,800
11 mm. larvae...	55	0.2	0.4	0.2	37.3	0	59.2	0.2	2.5	5,500
12 mm. larvae...	41	2.0	2.0	0.7	40.9	0	53.8	0.5	0.1	4,100
13 mm. larvae...	23	0.6	0.7	0.9	45.8	0	51.3	0.6	0.1	2,300
14 mm. larvae...	48	0.6	1.5	0.3	36.1	0	57.9	1.5	2.1	4,800
15 mm. larvae...	70	0.2	0.5	0.9	54.7	0	41.2	2.3	0.2	7,000
16 mm. larvae...	41	0.7	0.7	0.2	39.1	0	58.3	0.5	0.5	4,100
17 mm. larvae...	38	0.6	1.5	0.3	33.1	0	63.2	0.3	1.0	3,800
18 mm. larvae...	21	1.6	5.0	0.3	35.3	0	57.4	0.0	0.4	2,100
19 mm. larvae...	93	0.4	1.2	0.6	42.3	0	53.7	0.5	1.3	9,300
20 mm. larvae...	58	0.3	1.4	0.4	38.6	0	58.7	0.4	0.2	5,800
21 mm. larvae...	44	0.7	2.7	0.5	33.8	0	60.4	0.3	1.6	4,400
22 mm. larvae...	53	0.9	3.1	0.5	33.4	0	60.9	1.0	0.2	5,300
23 mm. larvae...	37	0.7	1.9	0.9	37.6	0	56.9	1.9	0.1	3,700
24 mm. larvae...	63	0.2	0.7	0.4	40.4	0	57.0	0.7	0.6	6,300
25 mm. larvae...	37	0.2	0.6	0.2	41.9	0	56.6	0.2	0.3	3,700
26 mm. larvae...	49	0.2	1.1	0.6	34.2	0	62.7	1.1	0.1	4,900
27 mm. larvae...	37	0.2	0.8	0.2	47.1	0	50.7	0.7	0.3	3,700
28 mm. larvae...	36	0.4	1.3	1.5	38.4	0	57.5	0.8	0.1	3,600
29 mm. larvae...	37	0.2	1.2	1.1	38.8	0	55.8	2.8	0.1	3,700
30 mm. larvae...	58	0.1	0.7	1.4	45.1	0	52.2	0.4	0.1	5,800
31 mm. larvae...	1	0.4	0.6	0.3	31.0	0	67.1	0.1	0.5	800
33 mm. larvae...	1	0.0	0.0	0.0	43.1	0	56.8	0.1	0.0	1,000
1–24 hrs. pupae..	24	0.4	0.8	0.3	46.7	1.0	47.9	0.9	2.0	4,000
2–4 day pupae...	16	0.1	0.5	0.4	46.2	0	49.6	1.0	2.2	1,600
5–7 day pupae...	4	0.1	0.8	1.3	51.2	0	44.3	0.0	2.3	2,500
1–24 hrs. adults..	17	0.2	0.1	1.1	38.9	0	57.6	1.4	0.7	1,700
25–144 hrs. adults	68	0.1	0.2	0.8	43.6	0	54.8	0.3	0.2	6,800
185 hrs. adults...	33	0.1	0.1	0.1	49.6	0	49.4	0.2	0.5	3,300
Total: 1,364										Total: 142,400

* Roman numerals refer to the 8 classes as named on p. 356; most are illustrated in figure 1.

they are absent in certain other adult insects which possessed them in earlier stages.

The hemocytes of the mealworm have been studied by three investigators. Ries (3), in studies of mycetome transplants into the hemocoel of various insects, considered that all of the hemocytes of the mealworm were fundamentally the same and referred to them all as "lymphocytes."[3] Although he described the response of the hemocytes to transplanted material, he did not make differential counts, nor did he categorize them. Nevertheless, his illustrations depict nuclear and cytoplasmic differences among the cells. Rooseboom (4) identified macronucleocytes, micronucleocytes, granular leucocytes, and oenocytoids in mealworm larvae, but she did not give differential counts for them. Jackson (1) studied the histogenesis and morphogenesis of the hemocytes of mealworm larvae and adults. He classified the cells as macronucleocytes, micronucleocytes, and oenocytoids. In addition, he presented differential hemocyte counts showing 51.4 per cent macronucleocytes, 47.3 per cent micronucleocytes, and 1.3 per cent oenocytoids. The macronucleocytes and micronucleocytes of Jackson (1) appear to be partially comparable to the plasmatocytes and the cystocytes (coarsely granular hemocytes), respectively, of the present study. Jackson (1) did not present hemocyte counts from pupae since he was unable to secure satisfactory hemolymph smears from them. Although Jackson (1) implied a polyphyletic embryonic origin for his three hemocyte classes, the author has gained the impression from his observations that a monophyletic origin for the hemocytes of the mealworm seems more likely.

SUMMARY AND CONCLUSIONS

1. With a few modifications, the hemocytes of the yellow mealworm, *Tenebrio molitor*, may be categorized according to Yeager's hemocyte classification (1945) for the southern armyworm, *Prodenia eridania*, even though the hemocyte picture, as a whole, of *Tenebrio* is not as complex as that of *Prodenia*.

2. Eight hemocyte classes of the mealworm have been identified and differentially counted from representative ages throughout the larval, pupal, and adult stages of this insect. The eight classes are prohemocytoids, smooth-contour chromophilic cells, oenocyte-like cells, plasmatocytes, vermiform cells, cystocytes (or coarsely granular hemocytes), spheroidocytes, and degenerating cells.

3. Twenty-three sub-types of hemocytes are designated as making up the eight main classes. Dimensional ranges for the nucleus and the entire cell of these sub-types are given in Table 1.

4. Neither podocytes nor eruptive cells, as they are described for *Prodenia*, occur in the peripherally circulating hemolymph of the mealworm.

[3] Ries (3) stated that he planned to discuss the hemocytes of the mealworm in a later paper, but such a publication has not, as yet, come to the attention of the writer.

5. Plasmatocytes and cystocytes (coarsely granular hemocytes) make up more than 90 per cent of the hemocytes encountered in the normal hemolymph of the mealworm, but the other cells classes make up an essential part of the normal hemocyte picture.

6. The various hemocyte classes appear to be derived from prohemocytoids, with the possible exception of the oenocyte-like cells.

7. Neither multinucleate nor binucleate hemocytes were seen in the 142,400 hemocytes counted.

LITERATURE CITED

1. JACKSON, H. W.
1939. The morphology and histogenesis of the blood of the mealworm (*Tenebrio molitor* L.) with observations on its embryology. Doctor's dissertation, Cornell University Library, Ithaca, N. Y., 111 p.p. Illus.

2. PEREZ, C.
1910. Recherches histologiques sur la métamorphose des muscides *Calliphora erythrocephala* mg. Arch. Zool. expér. et gen. 4: 1–274.

3. RIES, E.
1932. Experimentelle Symbiosestudien. I. Mycetomtransplantationen. Zeit. wiss. Biol. (A) 25: 184–234

4. ROOSEBOOM, M.
1937. Contribution á l'étude de la cytologie du sang de certain insectes avec quelques considerations generales. Arch. Néerl. Zool. 2: 432–559.

5. YEAGER, J. F.
1945. The blood picture of the southern armyworm (*Prodenia eridania*). Jour. Agr. Res. 71:1–40.

AUTHOR INDEX

SUBJECT INDEX